STUDIES ON THE KINETICS OF ADDITION-ELIMINATION TYPE INTERACTIONS

ABSTRACT

THESIS

SUBMITTED FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy

IN

CHEMISTRY

BY

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DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

2002

ABSTRACT

The carbonyl group $>C=O$ is a structural feature of many different types of compounds. There are carbonyl compounds ranging from aldehydes and ketones to carboxylic acids and their derivatives. Substances with amine functionality react with aldehydes or ketones by an addition-elimination sequence. Ninhydrin–α-amino acid reactions also have feature of general addition-elimination type interactions. The reaction produces a purple-coloured product, diketohydrindylidenediketohydrindamine (DYDA), popularly known as Ruhemann's purple.[1] Due to importance of the ninhydrin coloured-reaction in the analysis of α-amino acids in peptide chemistry, it became the focus of various structural and mechanistic investigations.[2] In earlier days, different workers encountered problems associated with estimation of α-amino acids by ninhydrin, e.g., reproducibility, intensity of colour, stability of colour, etc. Continuous efforts are, therefore, being made to improve the method of estimation of α-amino acids by ninhydrin.

Surfactants have some unique properties which make them attractive for chemical reactivity[3] and physico-chemical studies.[4] They have industrial applications as well.

Surfactant micelles provide different micro-environment for different parts of the reactant molecule, i.e., a nonpolar hydrophobic core can provide binding energy for similar groups while the outer charged shell can interact with the reactants polar groups. This inherent microheterogeneity of the micellar solubilization environment could play an important role in the catalysis of a reaction.

Due to its importance, systematic kinetic studies of the formation of DYDA between ninhydrin and some amino acids have, therefore, been performed under different experimental conditions.

The work described in this thesis titled, "Studies on the Kinetics of Addition-Elimination Type Interactions", is a systematic kinetic study of ninhydrin-amino acid reaction in aqueous, aqueous-organic and surfactant micellar media. The amino acids used were DL-alanine, DL-methionine, DL-threonine, L-tyrosine, L-glutamic acid and L-arginine.

The lay out of the thesis is as follows : (i) Chapter 1 - ***General Introduction***; (ii) Chapter 2 - ***Experimental***; and Chapter 3 - ***Results and Discussion***.

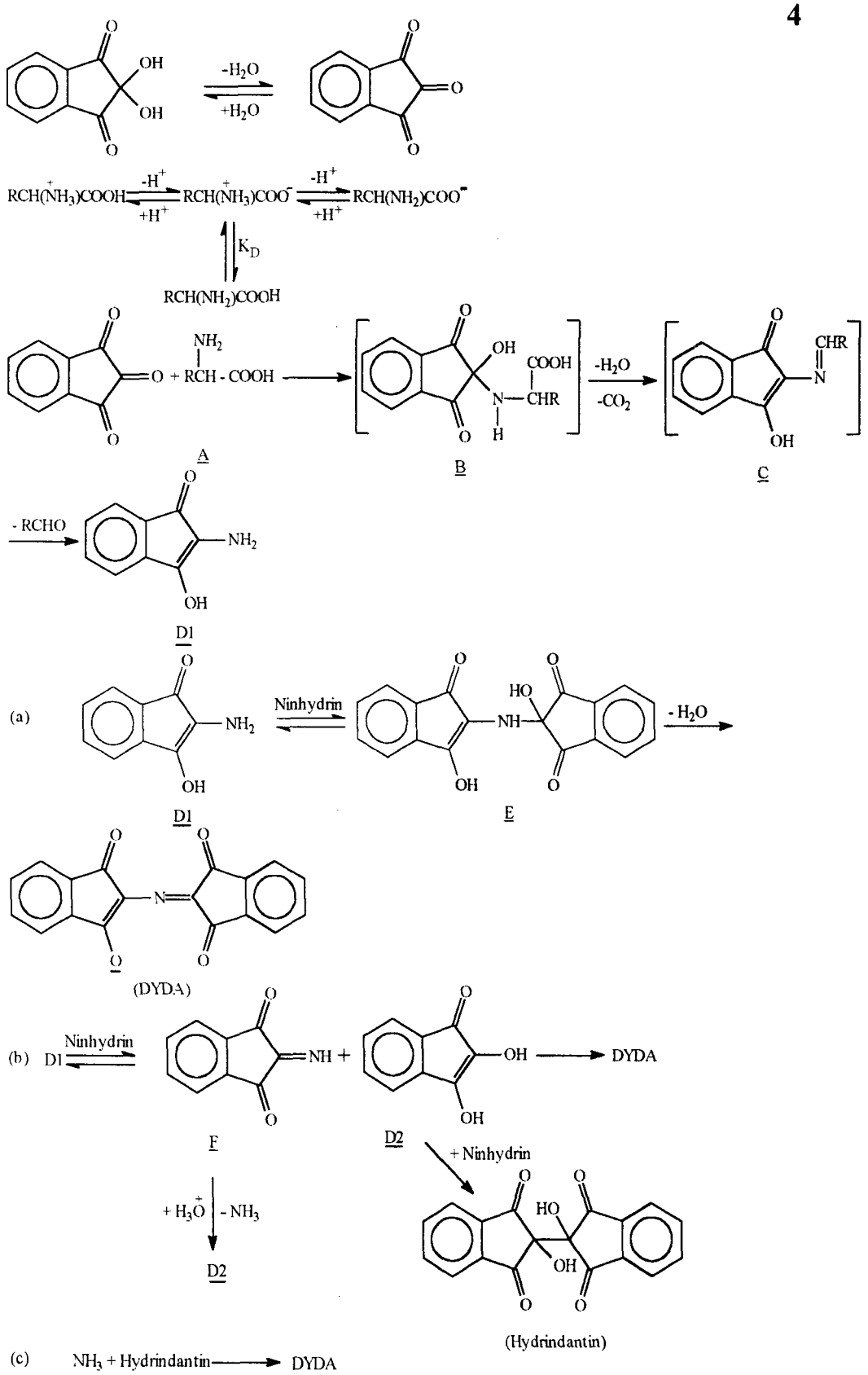
Chapter 1 comprises of details of ninhydrin-amino acids reaction mechanism in aqueous medium (that includes pertinent literature survey of the work performed on kinetic and mechanistic studies), introduction of surfactants, their solution properties, structural variations, pseudophase model of micellar catalysis and statement of the problem.

Chapter 2 provides experimental details. The source and purity of various reactants and surfactants are given. Procedure for the preparation of solutions, pH measurements, kinetic procedure details and determination of cmc under the reaction conditions have been given. Spectra of the reaction product obtained under varying conditions are also presented in Chapter-2. The product (diketohydrindylidenediketohydrinamine, DYDA) has λ_{max} at 400 and 570 nm.

As the wavelengths of maximum absorbance remained unchanged in the presence of solvents and micelles, 570 nm was selected as the wavelength to follow the kinetics.

Chapter-3 details different experimental conditions that were adopted. To elaborate their roles, different concentrations of amino acids, ninhydrin, surfactant (CTAB) and solvents (acetonitrile, dimethyl sulfoxide, methyl cellosolve, and 1-propanol) were used. It was observed that the values of rate constants were not dependent on the initial concentration of amino acids, in both the media, indicating a first order dependence of the rate of reaction on amino acid concentration. The non-linear dependence of k_{obs} and k_{ψ} vs. [ninhydrin] suggests fractional-order of reaction with respect to [ninhydrin]. Thus, it has been concluded that the dependence of rate of reaction on amino acid and ninhydrin in presence of CTAB is similar to that of aqueous medium. Therefore, it is inferred that the mechanism of aqueous medium is operating in micellar media too.

The experiments establish that the interaction of ninhydrin with α-amino acids involves two steps. The reaction initiates by the attack of lone-pair of electron of amino nitrogen to the carbonyl carbon (of ninhydrin) to form a Schiff base (C) in the first step (Scheme - 1). The Schiff base C contains a double -bonded nitrogen atom and decarboxylation is considered to take place through unionized acid, possibly *via* formation of an unionized chelated ring structure. The second

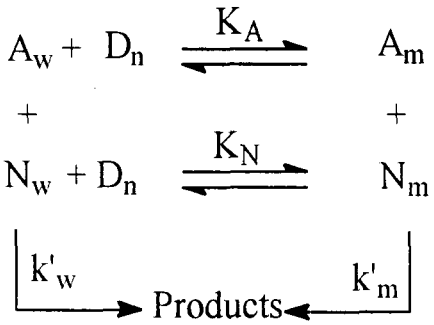


Scheme - 1

step involves the hydrolysis of C to D1 which, with ninhydrin, also involves an addition-elimination type interaction leading to the formation of DYDA.

The experiments were undertaken in the absence and presence of micelles such that reactions of alternative routes (b) and (c) were suppressed (almost completely) by performing studies at high temperatures ($\geq$ 80 °C) and pH 5.0 (formation of ammonia is negligible at pH $\geq$ 5.0).[5,6]

The effect of [CTAB] on the rate constants for ninhydrin-amino acid reactions are shown in Figs. 1-5. The reactions occurring in the presence of CTAB micelles follow Scheme - 2 :



Scheme - 2

The data have been treated by considering the distribution of reactants (amino acid, A, and ninhydrin, N) in aqueous and micellar *pseudo*-phases. Corresponding to the Scheme - 2, the following rate equation was obtained :

$$k_\psi = \frac{k_w [N]_T + (K_A k_m - k_w) m_N^S [D_n]}{1 + K_A [D_n]}$$

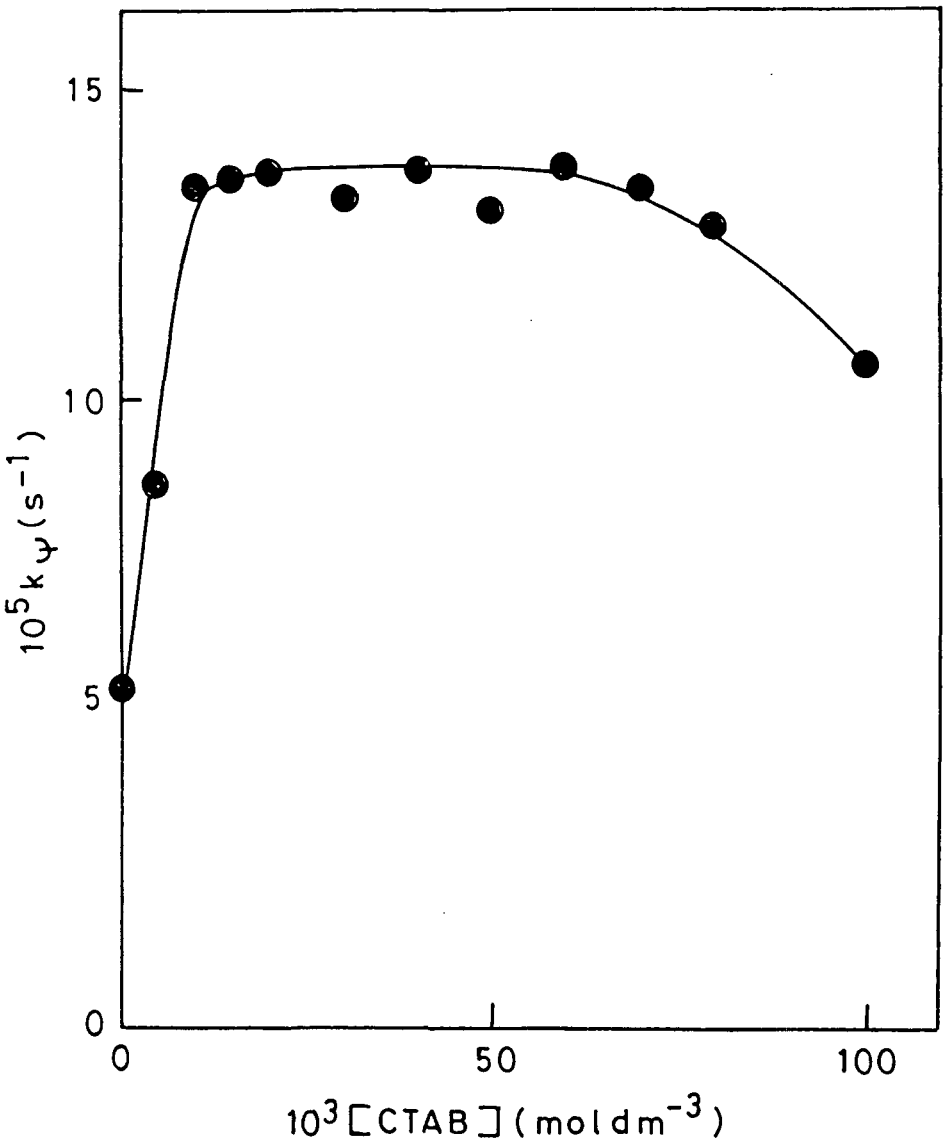


Fig. 1: Effect of [CTAB] on the reaction rate of alanine with ninhydrin. *Reaction conditions*: $[\text{alanine}]_T$ = 3.0 x 10^{-4} mol dm^{-3}, $[\text{ninhydrin}]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

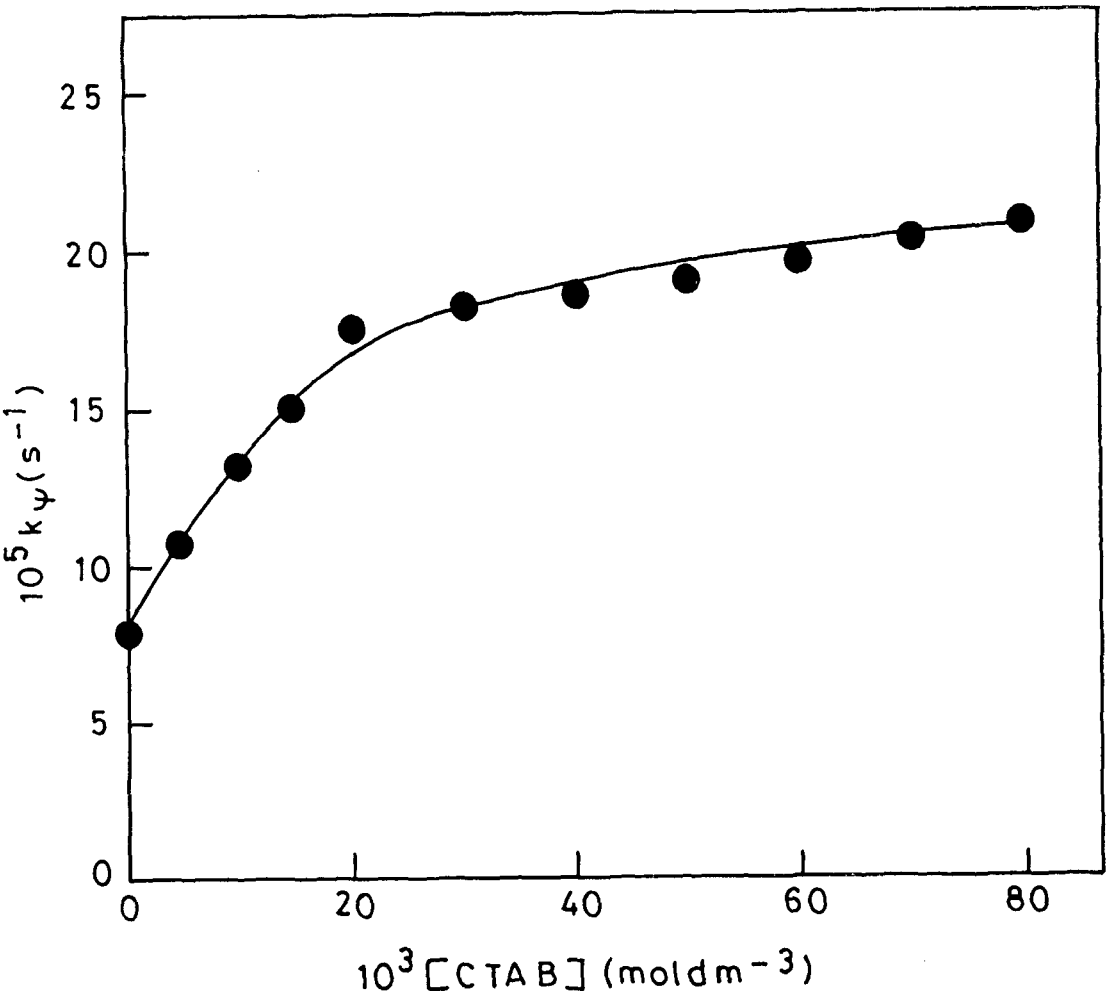


Fig. 2: Effect of [CTAB] on the reaction rate of threonine with ninhydrin. *Reaction conditions*: $[threonine]_T$ = 2.0 x 10^{-4} mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

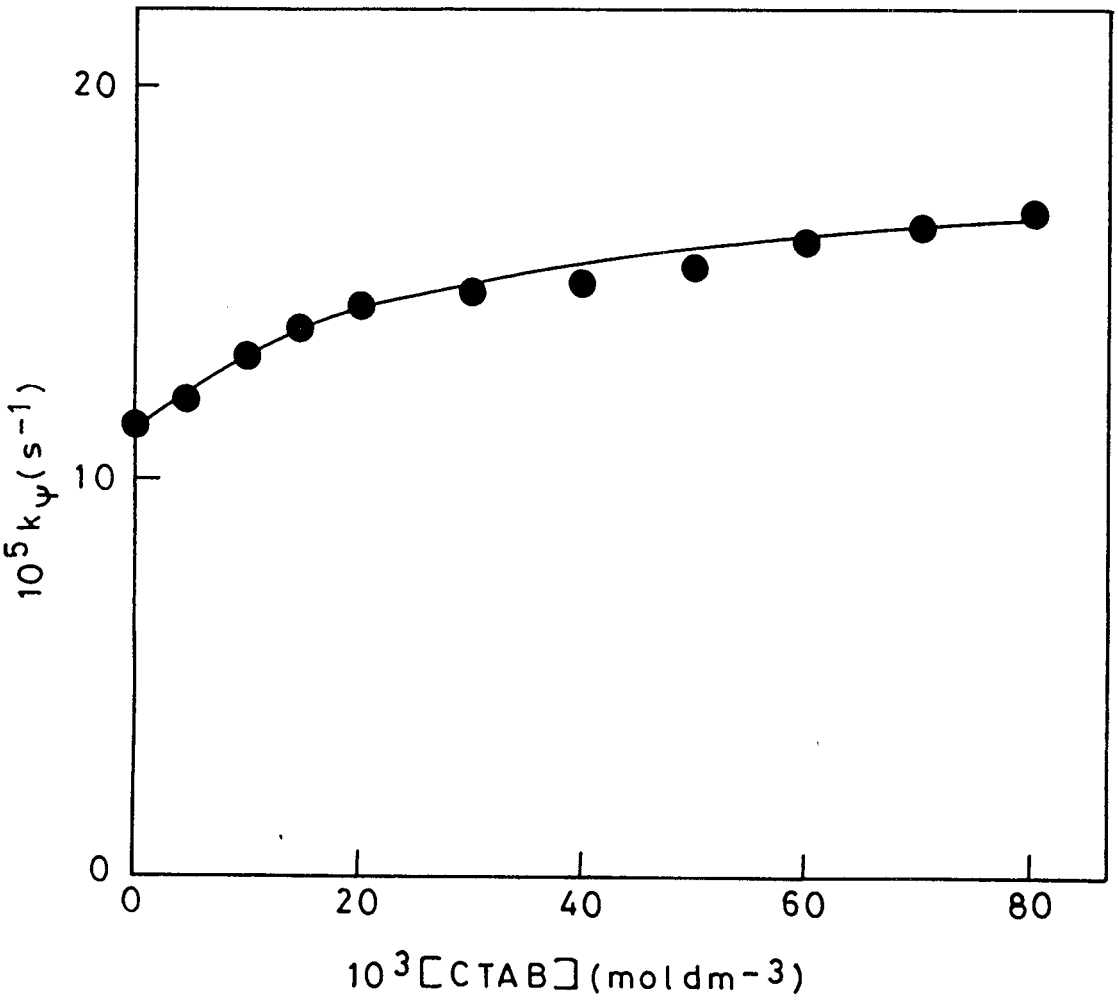


Fig. 3: Effect of [CTAB] on the reaction rate of tyrosine with ninhydrin. *Reaction conditions* : $[tyrosine]_T$ = 1.0 x 10^{-4} mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

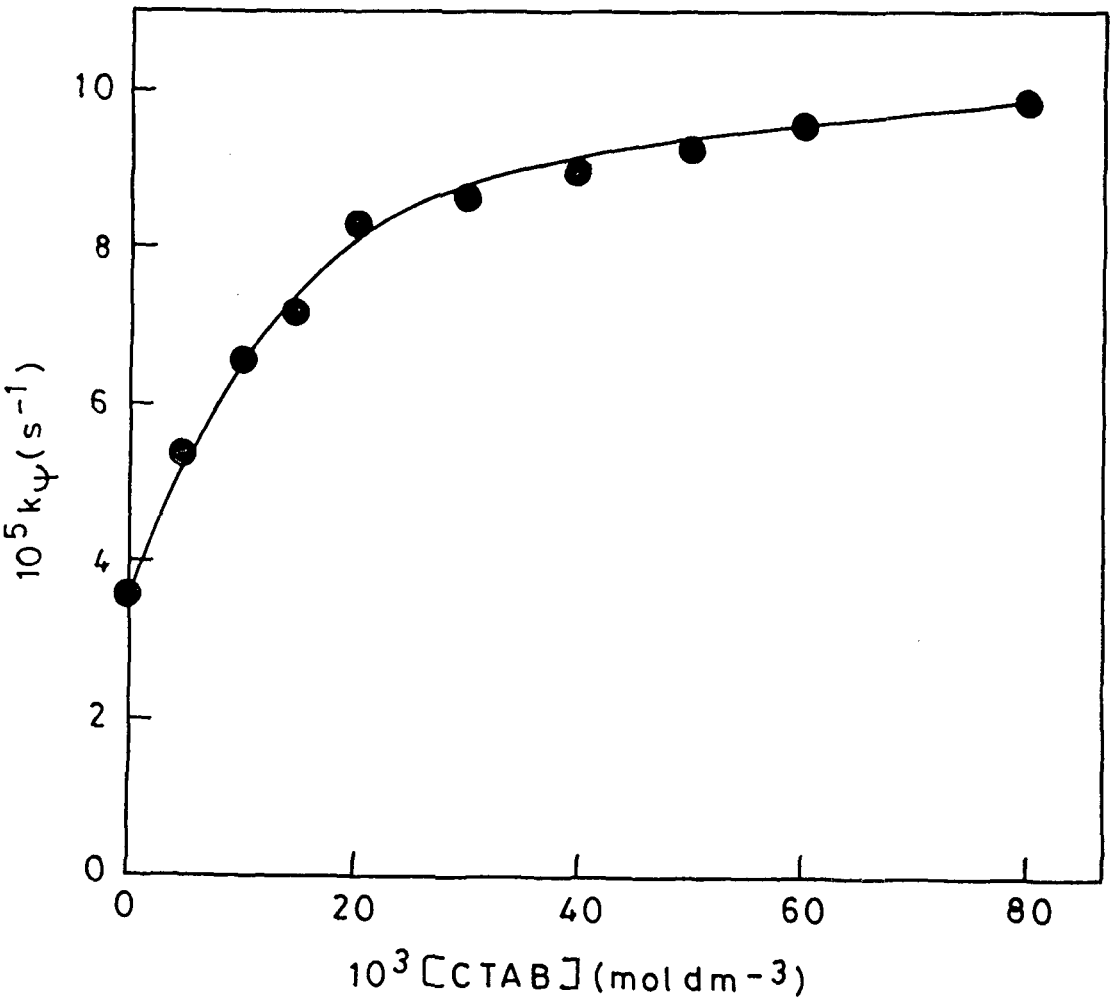


Fig. 4: Effect of [CTAB] on the reaction rate of glutamic acid with ninhydrin. *Reaction conditions* : $[\text{glutamic acid}]_T = 1.0 \times 10^{-4}$ mol dm^{-3}, $[\text{ninhydrin}]_T = 5.0 \times 10^{-3}$ mol dm^{-3}, pH = 5.0, temp. = 80 °C.

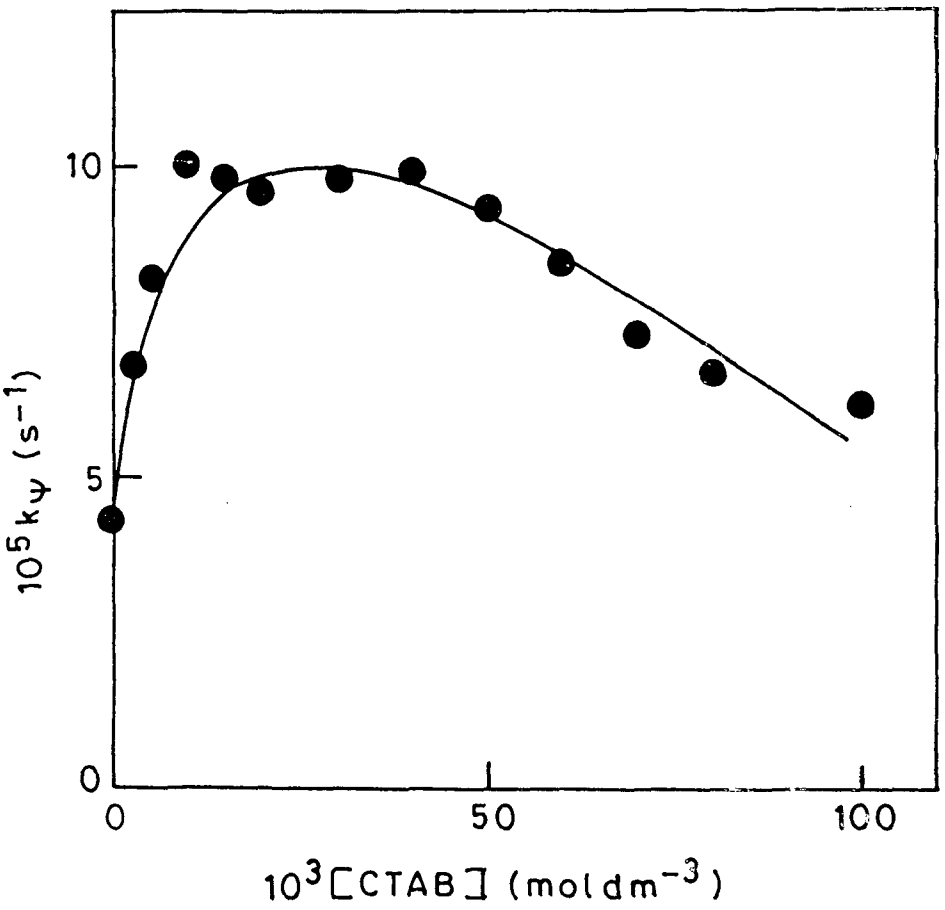


Fig. 5: Effect of [CTAB] on the reaction rate of arginine with ninhydrin. *Reaction conditions* : $[arginine]_T$ = 1.0 x 10^{-4} mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

Values of the second-order rate constants (k_m, s^{-1}) and binding constants (K_A and K_N) were obtained using a computer based program and are given in Table - 1.

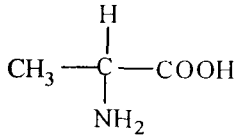
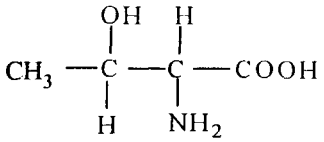
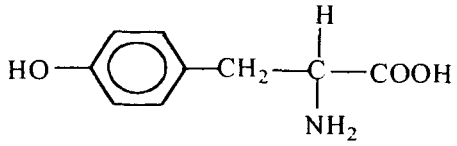
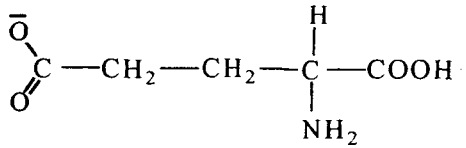
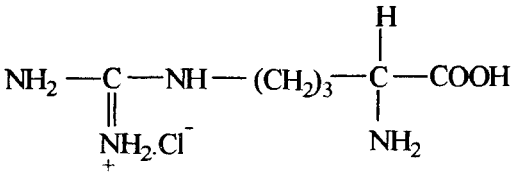
The increase in the rate of reaction in presence of cationic micelles could be attributed to the stabilization of intermediate C (Scheme-1) on the positively charged micellar surface which results in the increase of the concentration of the intermediate in the Stern layer. The presence of π-electrons in ninhydrin enhances the probability of its distribution between water and positively charged micelles. Therefore, the overall increase in rate of reaction is due to concentrating both the reactants in the Stern layer.

In order to evaluate activation parameters (E_a, $\Delta H^{\#}$ and $\Delta S^{\#}$), effect of temperature on the reaction rate was studied in absence and presence of CTAB. A comparative analysis of the values for both the media indicates that the presence of cationic micelles decreases the $\Delta H^{\#}$ and makes $\Delta S^{\#}$ more negative. This lowering occurs not only by virtue of the adsorption of both the reactants on the micellar surface but also because of stabilization of the transition state.

The influence of organic solvents on the ninhydrin reaction was studied at fixed [amino acid], [ninhydrin], and pH at 80 °C. Addition of organic solvents appreciably increase the rate as well as intensity of the colour. This unique behaviour of organic solvents towards the ninhydrin-

TABLE - 1

Kinetic results for the reaction of ninhydrin with amino acids in CTAB micelles at pH = 5.0, $[ninhydrin]_T = 5.0 \times 10^{-3}$ mol dm^{-3} and temp. = 80 °C

Amino acid	Structure	$10^3 k_m$ (s^{-1})	$10^4 k_2^m$ ($mol^{-1} dm^3 s^{-1}$)[a]	K_A ($mol^{-1} dm^3$)
Alanine		2.8	3.9	108
Threonine		5.6	7.8	82
Tyrosine		5.0	7.0	58
Glutamic acid		1.95	2.73	64
Arginine monohydrochloride		2.5	3.5	78

[a] $k_2^m = 0.14\ k_m$

amino acid reaction may be explained by the fact that as the solvent volume increases, the volume of water decreases in a given set of experiments, resulting in a decrease of the rate of hydrolysis (cf. Scheme - 1). Thus, with the increasing organic solvent content, the side reaction is progressively blocked. Secondly, Ruhemann's purple is highly soluble in organic solvents[5,7], thereby imparting increased intensity. A combined presence of DMSO and surfactant shows a synergistic effect, which could be due to blockage of side reaction and preconcentration of reactants in a small volume of the micellar surface region.

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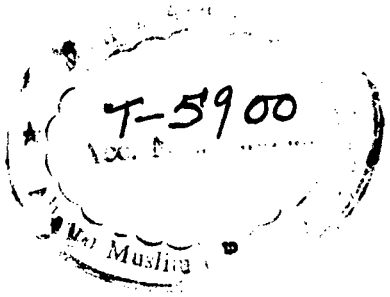

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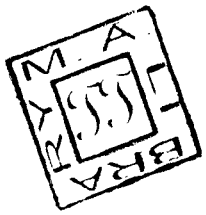

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Certificate

This is to certify that the thesis titled "**Studies on the Kinetics of Addition-Elimination Type Interactions**" is the original work carried out by **Mrs. Manzoora Bano** under my supervision and is suitable for submission for the award of **Ph.D.** degree in **Chemistry**.

Iqrar

(DR. IQRAR A. KHAN)

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Research is an organised and scientific analysis of things which cannot be accomplished without the co-operation and help of others. I have been extremely fortunate in this respect as I received generous help from all those to whom I approached. First of all my supervisor, namely **Dr. Iqrar A. Khan**, deserves special mention for his supervision which is unforgettable.

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It will be against my conscience not to mention the names of my parents, parents in law, sister and sisters-in-law, brothers-in-law namely, Gh. Rassol Mir (Advocate) and Fayaz Ahmad Mir (Lecturer) who provided me all possible help and co-operation and inculcated in me the sense of patience and appreciation of niceties of the present competititve world where excuses no more earn laurels.

I will be failing in my duty if I will not acknowledge the sincere and unprecedented co-operation extended to me by my husband. Research in any discipline unfolds untold problems for a researcher. These problems get multiplied if the researcher is married. However, my marriage has proved blessing in disguise for my research because I shared all the problems with my husband that not only eased out my tension but got ready made solutions. My husband being purely an academician, his love for learning and research inspired me and persuaded me to go for higher studies. My higher studies deprived my son, namely **Uzair Ibni Farooq**, of motherly love and affection. As a mother of one and a half years child, it was very hard decision to leave him and to go for research where only after the elapse of years next meeting with my child would have been possible. This does not, however, mean that I am absolving myself from

the responsibilities of a mother. I am committed to my son who only in the future will understand what his parents stand for.

In the last, but not the least, I offer my cordial thanks to all my teachers, colleagues and friends whom I could not mention by name.

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(Manzoora Bano)

LIST OF PUBLICATIONS

1. Kinetics and Mechanism of the Ninhydrin Reaction with DL-Methionine in the Absence and Presence of Organic Solvents.

   Kabir-ud-Din, **Manzoora Bano** and Iqrar A. Khan

   Indian J. Chem., Section B (in press).

2. Interaction between DL-Alanine and Ninhydrin in Aqueous and Aqueous-Organic Solvents and the Effect of Surfactant Micelles thereon.

   Kabir-ud-Din, **Manzoora Bano** and Iqrar A. Khan

   Indian J. Chem., Section A (communicated).

3. Reaction between L-Glutamic Acid and Ninhydrin : Role of Organic Solvents and CTAB Micelles.

   Kabir-ud-Din, **Manzoora Bano** and Iqrar A. Khan

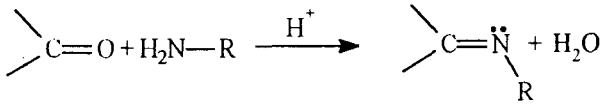
   J. Surface Sci. Technol. (communicated).

CHAPTER - 1

GENERAL INTRODUCTION

A. Addition-Elimination Reactions

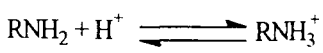
A wide variety of substances with $—NH_2$ groups react with aldehydes and ketones by an addition-elimination sequence to give $\rangle C{=}N—$ compounds and water. These reactions usually require acid catalysts :



(1.1)

Table 1.1 summarizes several important reactions of this type and the nomenclature of the reactants and products.

The dependence of the rates of these reactions on acid concentration is revealing with respect to mechanism and illustrates several important points relating to acid catalysis.



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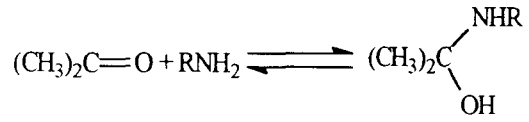

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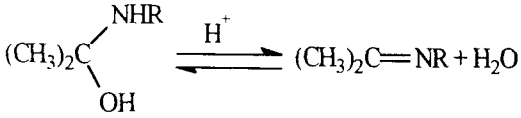


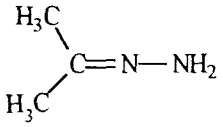
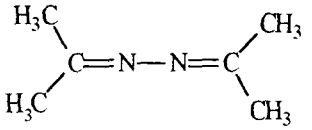
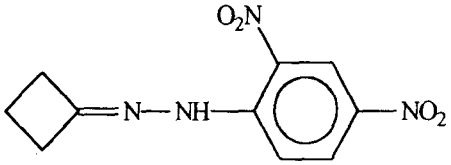
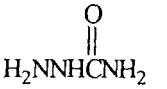
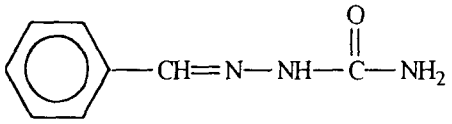
(1.4)

Scheme 1.1

Clearly, if the unshared electron pair on the nitrogen of RNH_2 is combined with a proton, (Eq. (1.2)), it cannot attack the carbonyl carbon

TABLE 1.1

Products from Reactions of Carbonyl Compounds with RNH_2 Derivatives

Reactant	Typical product	Class of product
$H_2N—R$ (R=alkyl, aryl, amine or hydrogen)	$CH_3CH=N—CH_3$	imine (Schiff base)
$H_2N—NH_2$ hydrazine		hydrazone
		azine
$H_2N—NHR$(R=alkyl,aryl) substituted hydrazine		hydrazone
 semicarbazide		semicarbazone
$HO—NH_2$ hydroxylamine	$H_2C=N—OH$	oxime

to give the aminoalkanol as in Eq. (1.3). So at high acid concentration (low pH) we expect the rate and the equilibrium for the overall reaction to be unfavourable. In more dilute acid, the rate picks up because there is more free RNH_2 in solution. Dehydration of the aminoalkanol (Eq. (1.4)) is acid catalyzed; this reaction normally is fast at pH values smaller than 3–4. Therefore, the slow step at pH < 4 is addition of RNH_2 to the carbonyl group as per Eq. (1.3). As the pH is increased above 4, the addition becomes progressively faster because less RNH_2 is tied up as RNH_3^+. However, then the dehydration step (Eq. (1.4)) decreases in rate because it requires an acid catalyst. At pH 6 (that means 100-fold decrease in H^+ concentration), dehydration is the slow step, and at higher pH values it finally becomes too slow to give a useful overall rate of reaction.

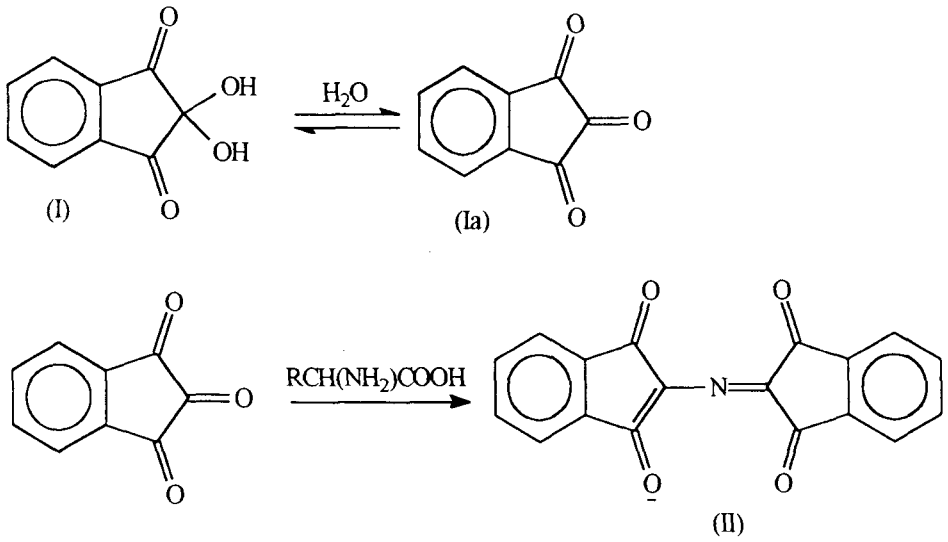
The colour-forming ninhydrin-α-amino acid reactions have characteristic of common addition-elimination type reactions. In the present thesis the work performed on the kinetics and mechanism of the reactions of some α-amino acids with ninhydrin are described.

Ninhydrin-Amino Acid Reaction and its Mechanism

Ninhydrin is the name given by Abderhalden and Schmidt[1] to 1,2,3 - triketohydrindene (Ia), which, because of its peculiar colour reaction with α-amino acids and amines, was used by them as indicator in the dialysis method for detection of the activity of specific ferments in the animal organism under pathologic conditions. Ninhydrin is also known as

Ruhemann's reagent (the reagent was first discovered by Ruhemann in 1910[2]) or more systematically as 2,2-dihydroxy-1,3-indandione (because, in presence of water, ninhydrin exists as its hydrate, I).

The application of ninhydrin for the detection and quantitative estimation of α-amino acids has been well established since its discovery. The interaction of ninhydrin with α-amino acids produces purple-coloured product, diketohydrindylidenediketohydrindamine (DYDA), popularly known as Ruhemann's purple (II)



The intensity of the colour of DYDA directly depends upon the quantity of α-amino acid present in the sample. In the beginning, it was found that the interaction of ninhydrin with amino acid does not give reproducible results, and therefore the estimation of α-amino acids is not accurate. To overcome this problem, a number of studies were undertaken. The focus of these studies was to (i) obtain reproducible results, (ii) stabilize

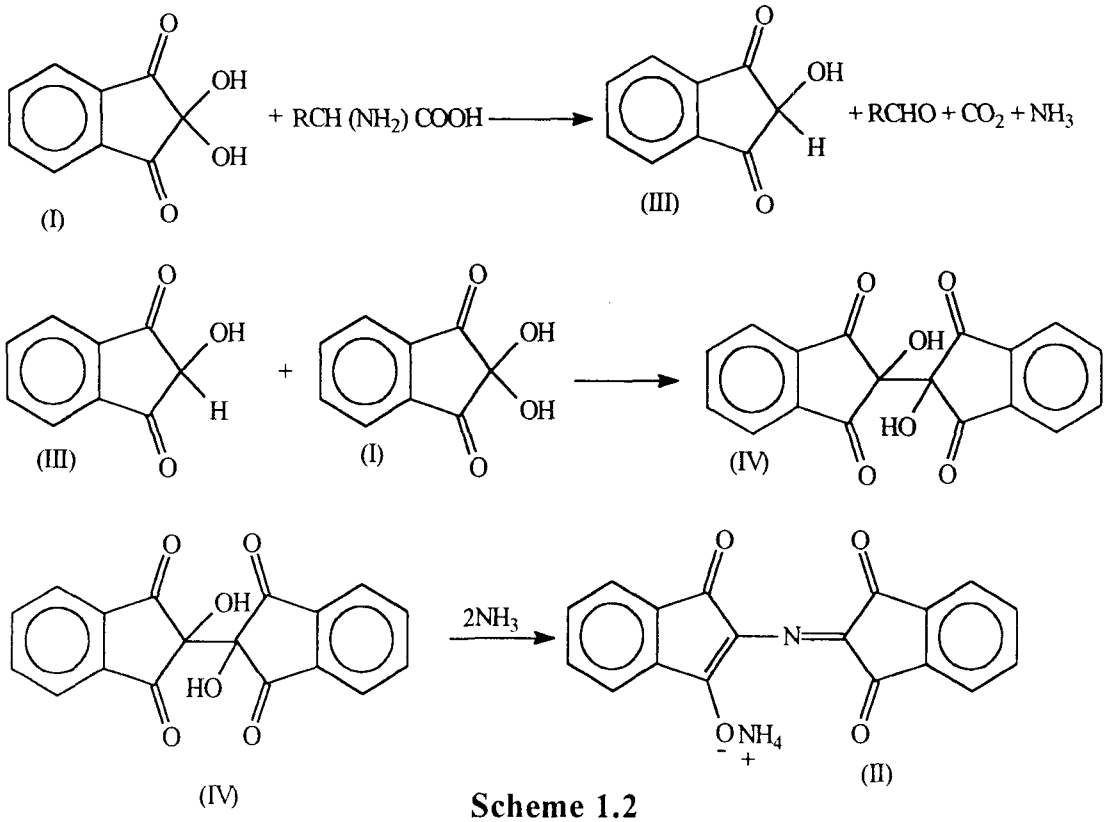
the coloured product, (iii) enhance the intensity of colour by addition of other reagents (e.g., organic solvents, reducing agents, etc.) to lower the detection limit and, (iv) identify intermediates to achieve the above goals in a proper way. Keeping these points in view, many researchers undertook mechanistic studies for ninhydrin-amino acid reaction and broadly divided the mechanism of the reaction in three steps:

(i) the initial attack of amino nitrogen of amino acid to carbonyl group of ninhydrin;

(ii) oxidation and reduction steps leading to intermediates along the pathway; and

(iii) formation of Ruhemann's purple from these intermediates.

Because of the significance of the ninhydrin reaction in the detection and quantitative estimation of α-amino acids in peptide chemistry, Ruhemann's purple became the focus of numerous structural and mechanistic studies. The very first attempt on mechanistic studies of ninhydrin-amino acid reactions was made by Ruhemann himself[2,3] (Scheme 1.2) but he failed to explain the formation of 2-hydroxy-1,3-indandione(III) and DYDA (Scheme 1.2). No doubt he observed that both amines and α-amino acids yield the purple colourded product but could not explain the formation of colour by amines. Ruhemann's original work[2] suggested the final step in DYDA formation to be the reaction of hydrindantin (IV) with two molecules of ammonia, derived from the

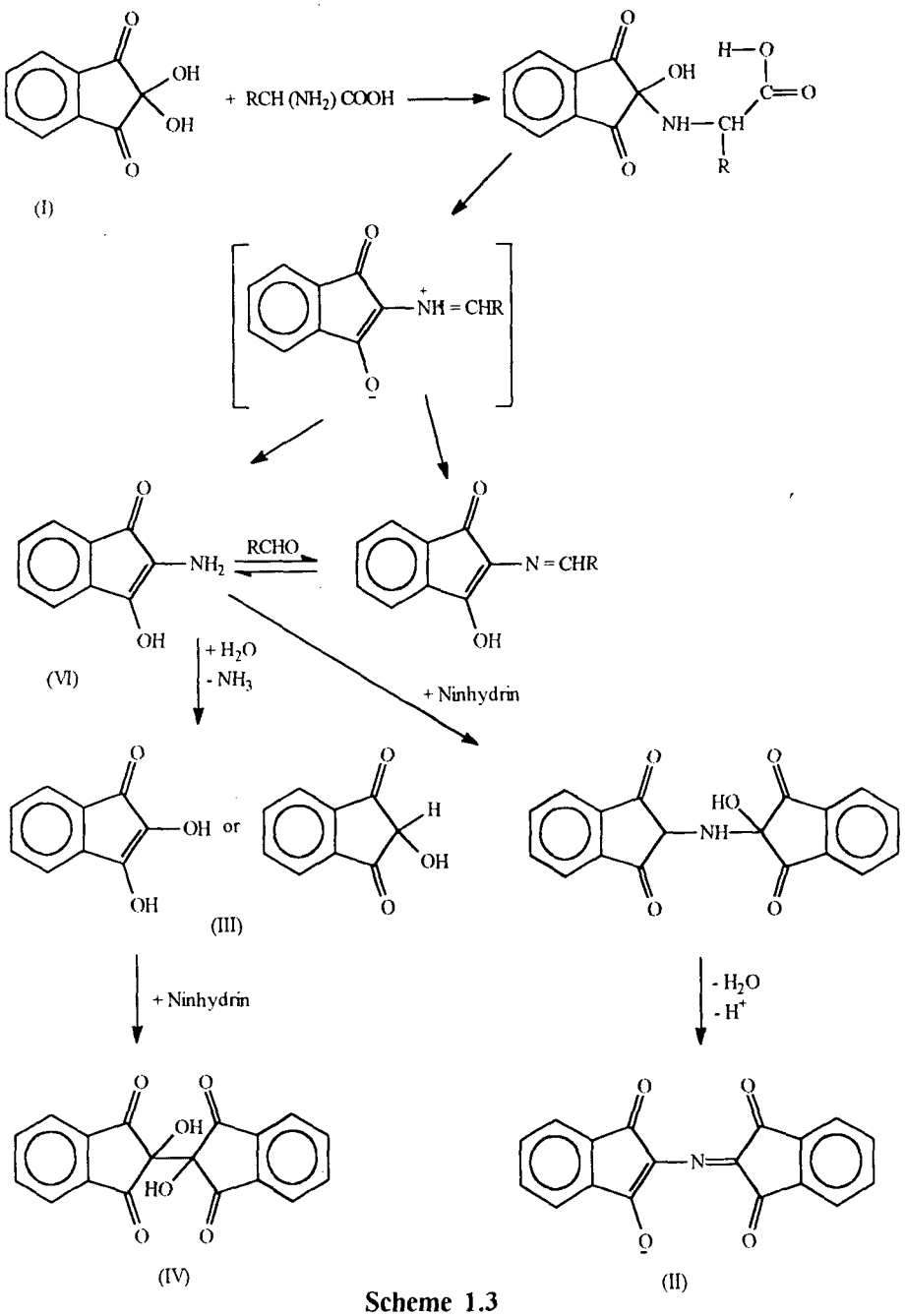


Scheme 1.2

amino acid. This mechanism cannot account for the observation that colour formation is decidedly more rapid with amino acids than with ammonium salts.[4,5]

This mechanism was elaborated by McCaldin.[6] The formation of hydrindantin was shown as a side reaction that was not directly involved in the colour forming pathway. The proposed mechanism (Scheme 1.3) also explained why amino acids react faster than amines and ammonia. In case of α-amino acids the adjacent carboxylate (to the amine group) loses CO_2, but in case of amines and ammonia, the cleavage of C-C or C-H bonds are involved.

The most thorough study of the role of hydrindantin was that of MacFadyen and Fowler[7]. The rate of disappearance of red colour of


+ RCH (NH2) COOH
(I)
NH = CHR
RCHO
NH2
N = CHR
(VI)
+ H2O
- NH3
+ Ninhydrin
OH or
(III)
+ Ninhydrin
- H2O
- H+
(IV)
(II)

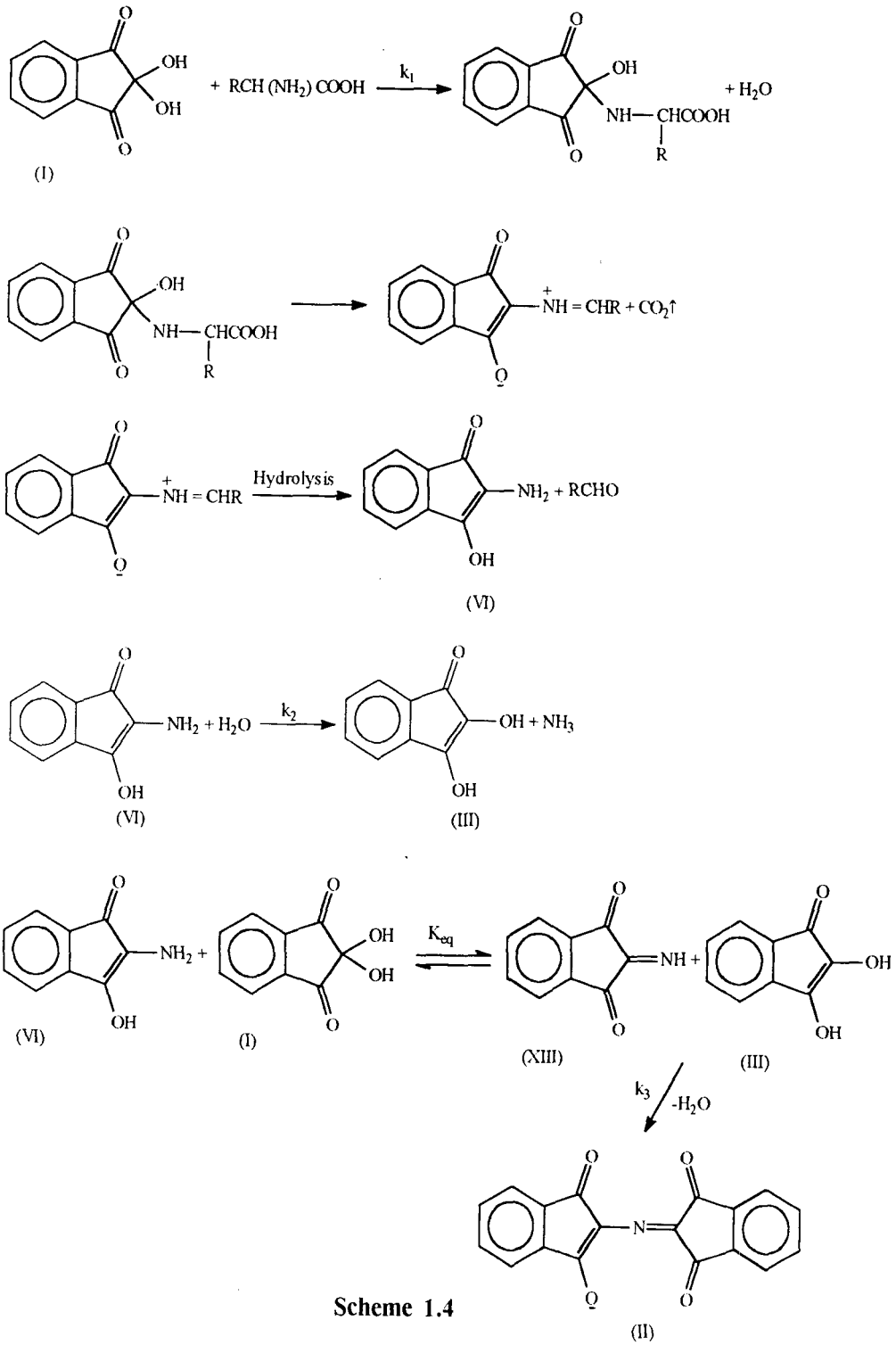
Scheme 1.3

hydrindantin was observed to be equal to that of appearance of purple colour for hydrindantin-α-amino acid reaction. It was suggested that hydrindantin hydrolyses to given 2-hydroxy-1,3-indanedione (III), and its one molecule is used for the formation of each molecule of Ruhemann's purple (II).

Based on their studies, Wittmann and co-workers[8] also suggested a role for hydrindantin in the formation of 2-hydroxy-1,3-indanedione (III), which could react with 2-amino-1,3-indanedione (VI) to give the purple-coloured product.

Lamothe and McCormick[9] explained the dependence of colour formation on hydrindantin concentration. They proposed a mechanism (Scheme 1.4) suggesting that α-amino acids react with ninhydrin to give VI and an aldehyde with one less C-atom than the original amino acid. The authors, on the basis of cyclic voltammetric studies, found that VI is a better reducing agent than ascorbic acid and is also unstable. VI may hydrolyse to give 2-hydroxy-1,3-indanedione (III) and NH_3 or react with ninhydrin to give 2-imino-1,3-indanedione (XIII) alongwith III. The last two react to give the Ruhemann's purple.

Friedman and Williams[10] considered the factors that affect the stoichiometry of formation of Ruhemann's purple. Their proposed mechanism is very similar to that of Lamothe and McCormick but does not incorporate the last step before the formation of Ruhemann's purple. They suggested that 2-amino-1,3-indanedione (VI) reacts with excess

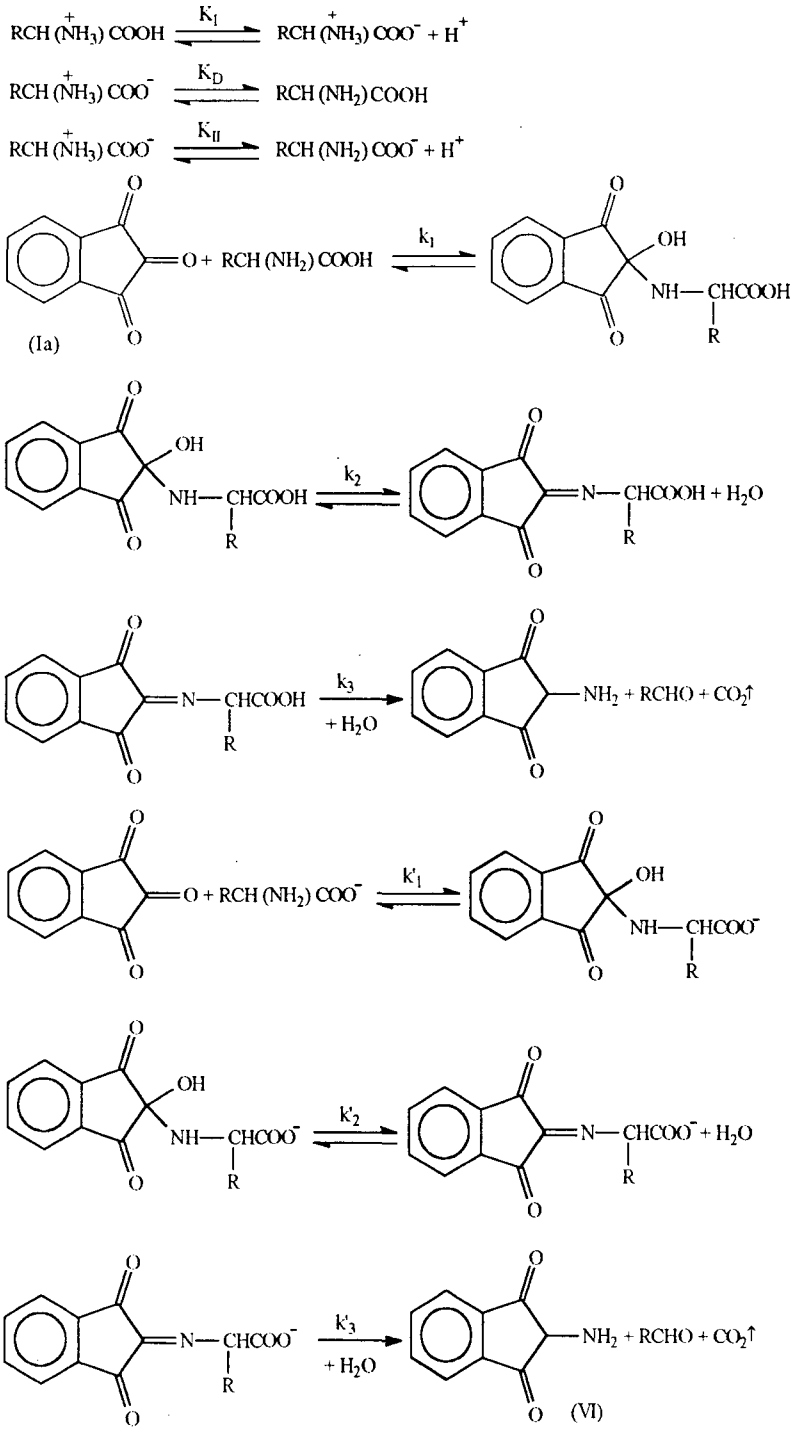


Scheme 1.4

ninhydrin rapidly to give the Ruhemann's purple. They also proposed an initial Schiff base condensation rather than nucleophilic displacement. They reported that at pH ≥ 4, excess ninhydrin gives the best colour yield for ninhydrin-amino acid reaction. At low pH, amines are protonated and does not behave as a nucleophile. At high pH, lone pair of electrons of amino group attacks the carbonyl group of ninhydrin to give a Schiff base. At low concentration of ninhydrin, 2-amino-1,3-indanedione (VI) is not trapped by ninhydrin and is hydrolysed to 2-hydroxy-1,3-indanedione (III), which reacts with ninhydrin to produce hydrindantin (IV, a side product) which decreases the colour formation.

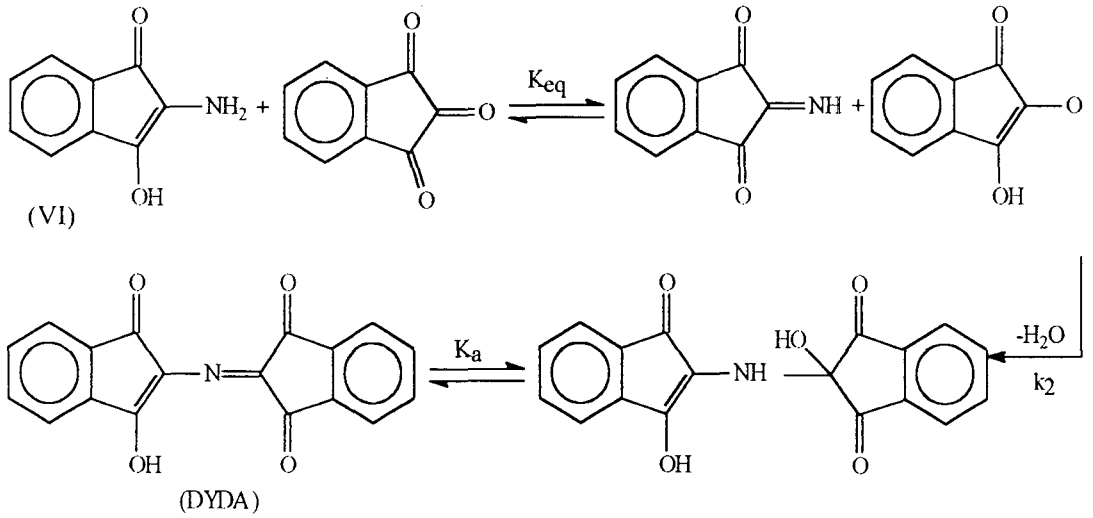
Bottom et al.[11] reviewed the possible mechanism for the formation of Ruhemann's purple and concluded that the one proposed by Friedman and William was the most consistent with the experimental findings but did not reject the conclusions of Lamothe and McCormick, who suggested that 2-imino-1,3-indanedione (XIII) and 2-hydroxy-1,3-indanedione (III) are the actual reactants in the Ruhemann's purple formation (cf. Scheme 1.5).

Khan and his associates[12-14] performed detailed kinetic and mechanistic studies on the ninhydrin-amino acid reactions at different pH, temperature, concentration of reactants and organic solvents. They found that the rate of evolution of CO_2 was faster than the rate of purple colour formation of DYDA. It was also observed that the evolution of CO_2 at 80 °C was complete within 20 minutes but, during the same time period, the



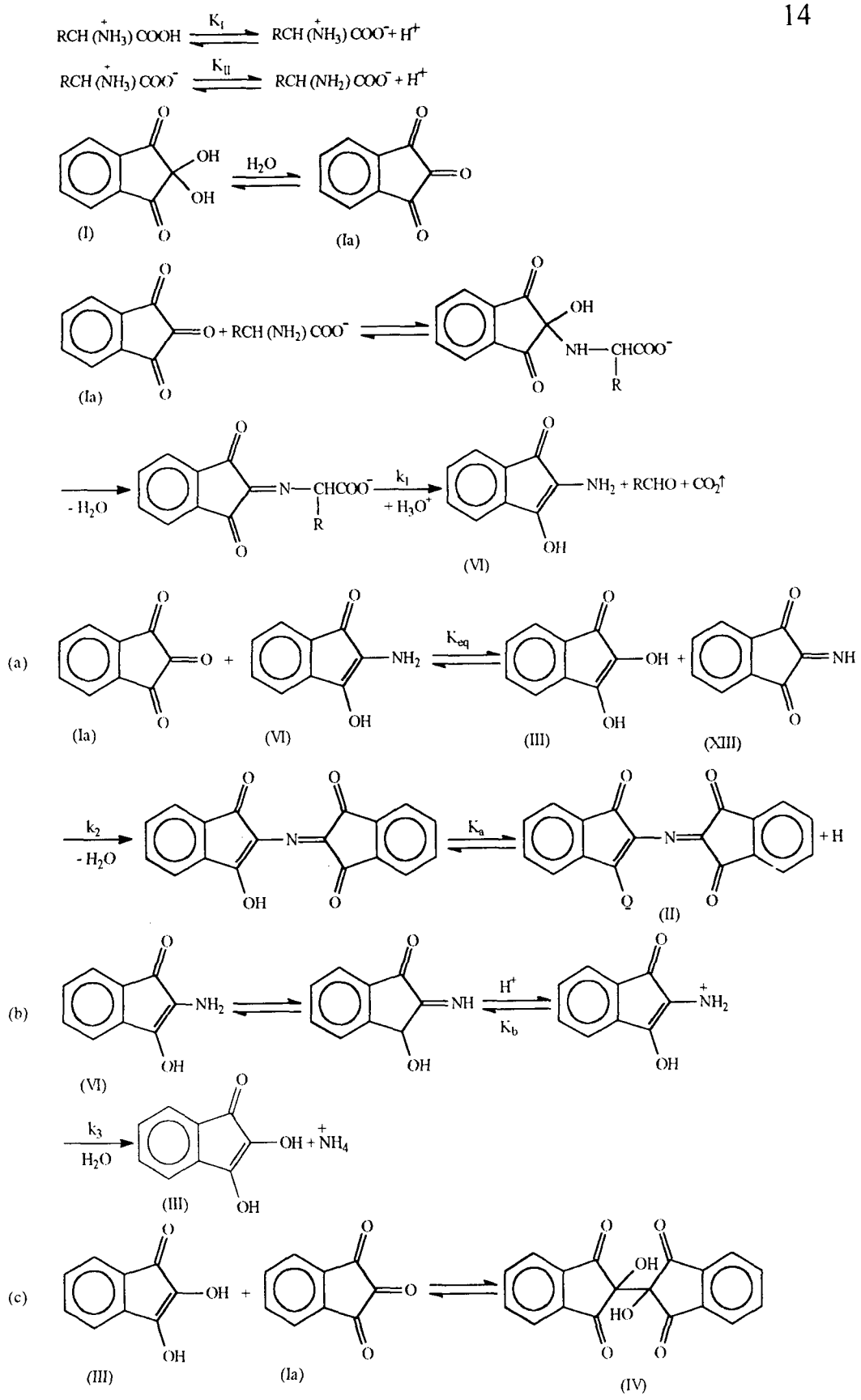
Scheme 1.5

intensity of purple colour was insignificant. They suggested mechanisms represented by Schemes 1.5 & 1.6 for the decarboxylation process as well as for development of the purple colour. Since the amino group of amino acids is protonated at low pH (and hence does not possess the free electron), they observed that nucleophilic attack of amino group of amino acid on carbonyl group of ninhydrin was not possible and decarboxylation did not take place. They further found that with the increase in pH, the equilibrium shifted towards unprotonated amine by which the rate for decarboxylation was increased. Consequently, they proposed a modified scheme by considering VI as a stable intermediate that acted as a reactant for the production of DYDA and, the conversion was considered as a three-step process, i.e.,



Accordingly, the following rate equation was established[12,13] for the formation of DYDA in aqueous medium at pH $\geq$ 5.0.

$$k_{obs} = K_{eq} k_2 K_a [N] / [H^+] \qquad (1.5)$$



Scheme 1.6

Kabir-ud-Din and coworkers,[15-20] in their studies on the kinetics of ninhydrin-amino acid reactions under *pseudo*-first-order conditions of excess ninhydrin, have explored the effect of micelles on the colour development. They found that cationic micelles increase the rate of purple colour formation with an increase in hydrophobicity of the amino acid side-chain.

B. Surfactants and Surfactant Micelles

In the last few decades, rapid progress has been made in exploiting the molecular organisation of amphiphilic molecules (e.g., surfactants). The word surfactant does not appear in most dictionaries. This is because it is not only a technical term, but also a diminutive form of the phrase SURFace ACTive AgeNT. The meaning now becomes a little clearer. Surfactants, also called surface active agents, detergents, tensides, are amphiphilic materials which contain both apolar, hydrophobic (lipophilic) and polar, hydrophilic (lipophobic) groups.[21-28] Surfactants are materials that tend not only to accumulate at surfaces, but which, by their presence, change the properties of those surfaces. Being surface active means that these molecules adsorb at the interface between two bulk phases, such as air and water, oil and water, or electrode and solution. The driving force for adsorption is the lowering of interfacial tension, i.e., minimization of interfacial free energy.[29]

In solvents which have a strong three-dimensional structure, e.g., water, hydrazine, 1,2-diols[30-33], or sulfuric acid[34], the dual character of the

amphiphile leads to self-association or micellization. Surfactants have polar or charged head groups and non-polar regions in the same molecule.[35] The basic building blocks of surfactants are derived from petrochemical and oleochemical (vegetable and animal) feedstocks. The latter are often referred to as "renewable resources" as opposed to petroleum-derived materials that are not considered to be renewable.

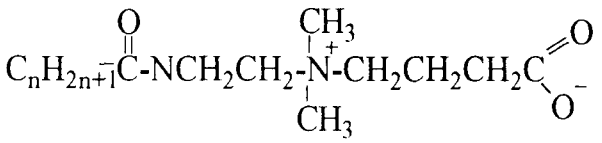
A surfactant, in general, can be ionic or nonionic depending on whether it ionizes in water or not. The hydrophobic group is usually a long chain hydrocarbon and the hydrophilic group is an ionic or highly polar group. Depending upon the nature of the hydrophilic group, the surfactants may be further classified as (i) cationic, (ii) anionic (iii) zwitterionic, and (iv) nonionic.

(i) *Cationic*, e.g., cetyltrimethylammonium bromide (CTAB), $CH_3(CH_2)_{15}N^+(CH_3)_3Br^-$; phenothiazine hydrochlorides, $C_6H_4(S)(NR)C_6H_3R'$

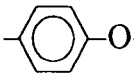
(ii) *Anionic*, e.g., sodium dodecyl sulfate (SDS), $CH_3(CH_2)_{11}OSO_3^-\,Na^+$; sodium perfluorooctanoate, $CF_3(CF_2)_6COO^-\,Na^+$

(iii) *Zwitterionic*, e.g., N-dodecylaminobenzylphosphinic acid,

$CH_3(CH_2)_{11}N^+H_2CH(C_6H_5CH_2)PO_2^-H$; amidoalkyl betaine,



(iv) *Nonionic*, e.g., N, N-dimethyldodecylamine oxide $CH_3(CH_2)_{11}\overset{\downarrow}{N}(CH_3)$; polyethylene glycol (*t*)-octylphenyl ether (TX-100), O

$(CH_3)_3CCH_2C(CH_2)_2$–  –$O(CH_2CH_2O)_{9.5}H$

The existence of two moieties in the molecule, one of which has affinity for solvent and other of which is antipathic to it, is termed as amphipathy.

When surfactant molecules are dissolved in water, they segregate their hydrophobic portion from water, by associating into a variety of aggregate structures, called micelles.[25,36-40] In aqueous solution the aggregation of amphiphilic molecules is largely due to hydrophobic interactions between the oleophilic parts of the molecules. As a consequence, there is a substantial free-energy change involved in the process of micellization which is due to the transfer of the oleophilic alkyl-chain part of the surfactant from an aqueous to a quasi-hydrocarbon environment. Aggregation of amphiphiles into micelles has been treated either as a stepwise association phenomenon or as a phase transition process.[41,42]

A great many studies have been published on the thermodynamics of micelle formation.[36,41-47] On the basis of some of these studies, it is now well established that a satisfactory understanding of the thermodynamics of micelle formation is vital in order to be able to describe quantitatively the more complex systems, such as bilayers and membranes. In view of

this, it is thus important that the theories on micelle formation be as consistent as possible.

The dynamic studies of the micelle have attracted special interest for the reason that the investigation of its physico-chemical properties is important and unavoidable in order to make clear the representative characteristics of micelles such as solubilization or catalysis. Further, the micelle has also been noted as a model of the biomembrane or the globular proteins, which is considered to have a structure similar to that of the micelle.

Although the equilibrium properties of the micelle have been studied to some extent, no kinetic studies have been performed until the development of the fast reaction techniques which have enabled kinetic investigations of fast reactions with half time down to about 10^{-9} s. The kinetic studies of micellization have been made on many surfactant systems by means of the various relaxation techniques.[48-57]

For micelles in water, the process of micellization is generally explained in terms of hydrophobic interactions between water and the surfactant. "Hydrophobic interactions" is in many ways a convenient term that is used to describe an entire array of inter- and intramolecular interactions involved in micellization and in a certain way, the term disguises our ignorance about the actual molecular dynamic processes that take place.[32,58] The formation of micelles in water is believed to take

place by association of the hydrophobic parts of the surfactant molecules and the repulsion of the water molecules from their immediate environment. The overall process of micellization involves a decrease in the free energy of the system. For aqueous solutions it is generally regarded as an entropy-directed process; the preponderant contribution of the entropy term being explained by disordering of the water structure and the breakup of the "Frank-Evans icebergs"[59] by the surfactant molecules.

In polar solvents such as water, monomers assemble to form a micelle in such a way that their hydrocarbon tails huddle in the core of the micelle, and the polar head groups project outwards into the polar bulk solution and locate at the micelle-water interface such that the hydrophobic tails are shielded from water (Figure 1.1). The charged head groups and the relatively small counterions of the ionic micelle are located in a compact region, known as the Stern layer, which extends from the core to within a few angstroms of the shear surface of the micelle. The compactness of the Stern layer is responsible for the reduction of the net charge on the micelle. Most of the counterions are, however, located outside the shear surface in the Gouy-Chapman electrical double layer where they are completely dissociated from the charged aggregate and are able to exchange with ions in the bulk of the solution (Fig.1.1). The amount of free counterions in the bulk solvent is expressed as the fraction of charge. The affinity of the micelle for the counterion is entropy controlled,[60] however entropy might not be the dominant factor at for all

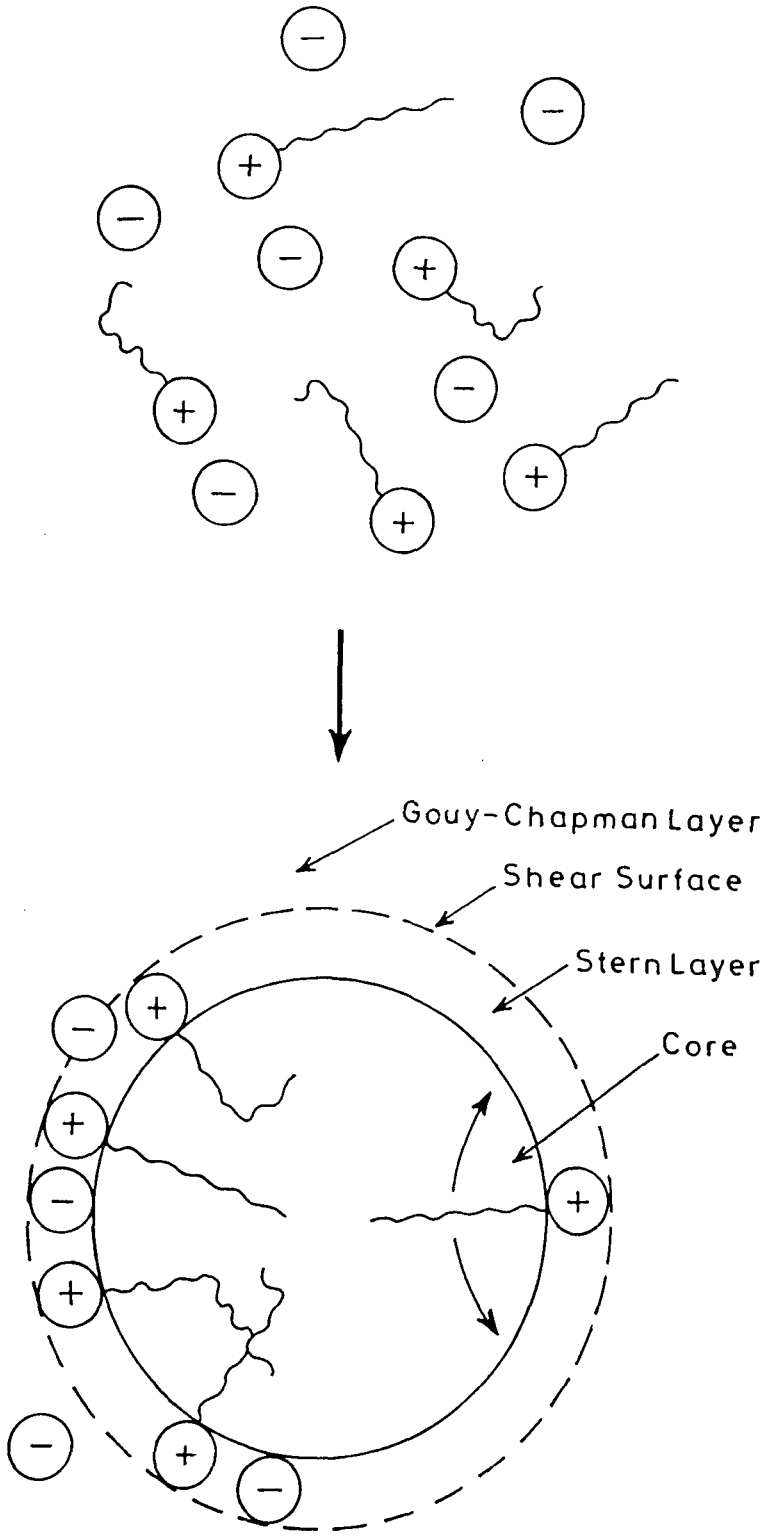


Fig. 1.1: Model of hypothetical cationic micelle showing the locations of head groups, surfactant chains and counterions. Curved arrows symbolize the liquid–hydrocarbon – like nature of the core.

counterions. These properties of the Stern layer are of key importance to the kineticist, and various probes have been devised for their study.

The kind of interactions occurring in the formation of micelles in polar solvents other than water are called solvophobic. The understanding of solvophobic interactions and micellization in non-aqueous and mixed aqueous media is considerably more nebulous.[58] The driving force for micellization in such systems is less than that for water and the increase in ΔG^{o}_{mx} is believed to be mainly the result of a decrease of the entropic contribution. Few solvent systems are as highly ordered as water. When amphipathic molecules aggregate, several biologically significant changes occur in the system of which they constitute a part: the concentration of monomeric species may increase only slowly or may decrease with increase in total concentration and the transport and colligative properties of the system are changed. If the amphipathic molecules have an intrinsic biological activity, then the formation of aggregates might well lead directly to an alteration in biological activity. One can envisage the aggregation of naturally occurring molecules leading directly to a change in biological activity due to decreased transport rates or decreased ability to pass through biological barriers. Or the ability of the aggregated species to interact with other biological species may change and there may be a physical alteration in the environment caused by micelle formation.

Aggregates can also form in apolar solvents. In such cases head groups of surfactant molecules locate inside to form a polar core and

hydrocarbon tails are directed towards the bulk solvent, to form the outside shell of the micelle. These are called reversed (reverse) or inverted (inverse) micelles. If there is any water in the medium, it will be entrapped in the core. This surfactant solubilized water is often referred to as a water pool and reverse micelles are sometimes called microemulsions. They are able to solubilize relatively large amount of water in their cores and this enables them to solubilize water soluble substances in non-polar solvents. They are also reported to form in near- and supercritical fluids.

The amphipathic molecules have a tendency to collect at any interface where the hydrophobic groups can be partially or completely removed from the contact of water and the hydrophilic groups can remain wetted. These general tendencies account for their surface activity, i.e., the ability to adsorb to air-water or oil-water interfaces and to surfaces of hydrophobic solids such as carbon or to macromolecules such as proteins. The same dual tendencies and the built-in asymmetry of the molecules allows them to form organized structures such as soap films and bilayers. The formation of lipid membranes by relatively insoluble amphipaths involves similar forces and structural features also.

Critical Micelle Concentration

Many, but not all, amphiphiles and surfactants form micelles. There is a relatively small range of concentrations separating the limit below

which virtually no micelles are detected and the limit above which virtually all additional surfactant forms micelles. Many properties of surfactant solutions (Fig. 1.2), if plotted against the concentration, appear to change at a different rate above and below this range . By extrapolating the loci of such a property above and below this range until they intersect, a value may be obtained known as the critical micelle concentration (cmc). The cmc is of central significance in micellar solutions and one of the most easily obtainable and useful quantitative results about aqueous flexible chain surfactant systems.[61] It has numerous applications for the thermodynamics of micelle formation and for characterizing micellar solutions.[61,62] Nearly all of these depend upon attaching the proper significance and weight to the critical nature of the cmc. Indeed, the cmc is sharp enough to form the basis of a two-phase model of the micellar systems which seems to overemphasize the critical nature of the cmc.[63]

One of the most practical uses of the cmc is in the calculation of monomeric and micellar concentrations.[61,62] In many cases, the approximations that no micelles are present below the cmc and above the cmc, the change in monomer concentration is small enough to be negligible, are reasonably valid.[62,64] As micelles are generally not very surface active, a corresponding practical approximate significance of the cmc is that it is the equilibrium concentration where surface chemistry ends and colloid chemistry begins. Critical micelle concentration can be determined by different experimental methods.[61,65,66]

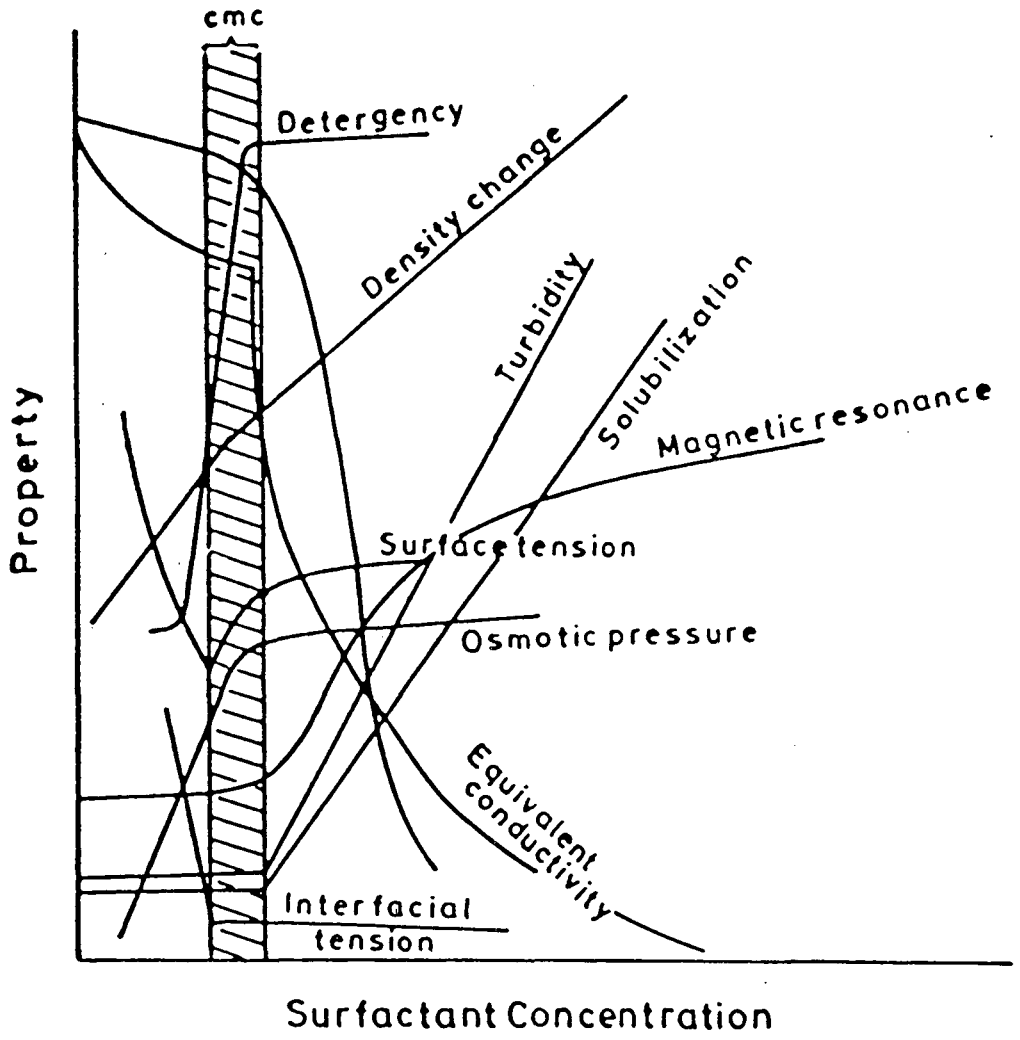


Fig. 1.2: Variation of physical properties with surfactant concentrations.

The cmc and solution properties depend upon the chemical structure of surfactants as well as physico-chemical conditions such as the concentration of added salts,[67,68] solvent polarity, temperatures, pH and pressure.[40]

Usually the more surface active the amphiphilic monomer, the higher is the tendency for micellization and hence, the lower the cmc of the amphiphile. Accordingly, the longer the total carbon chain length of the monomeric surfactant, the lower the cmc becomes. The number of carbon atoms, n, are empirically related to the logarithm of the cmc by

$$\log \text{cmc} = A - Bn \qquad (1.6)$$

where A and B are constants.[69,70]

The position of head group in hydrocarbon chain also affects the cmc. The closer the head group to the centre of the chain, the higher the cmc; due to the two branches of the chain partially shielding one another. The presence of double bond in the chain also causes an increase in cmc.

Additional polar groups, C=C double bonds, and chain branching tend to increase the cmc, but changes in the hydrophilic part of the amphiphilic generally have insignificant effects on the cmc. The addition of strong electrolytes reduces the cmc of ionic surfactants but only slightly alters that of nonionic surfactants. Non-polar solutes may also influence the cmc of all types of surfactants. Changes in the cmc as a function of temperature and pressure can provide thermodynamic data for micellization.[66,71]

Electrolytes that are known to be capable of 'salting-out' reduce the cmc of nonionic surfactants. Examples are NaCl, KCl, NaBr and $NaNO_3$[72] and the general rule is that small hydrated ions are more effective. Other electrolytes such as $Mg(NO_3)_2$ and $Al(NO_3)_3$ are capable of 'salting-in' nonionic surfactants.[73] Such salts have cations capable of forming complexes with the polyethylene oxide chains and increasing the level of hydration.

Where surfactant molecules contain ionisable groups such as $-NH_2$, $-(CH_3)_2N\rightarrow O$ and –COOH, the degree of the dissociation of the polar group will be very dependent on pH.[74] In general, cmc will be high at pH values where the group is charged (low pH for $-NH_2$ and $-(CH_3)_2N\rightarrow O$, high pH for –COOH) and low when uncharged. Some zwitterionic surfactants become cationic at low pH, a change that can be accompanied by a rapid rise in the cmc[75] or a more modest rise[76] depending on the structure and hence hydrophilicity of the zwitterionic form.

cmc is also affected by temperature. The cmc would at first sight be expected to increase as temperature increases, due to a thermal agitation decreasing adhesion between monomers, so shifting the equilibrium to favour the monomeric species. This is probably true for ionized surfactants at higher temperatures. At lower temperatures the cmc decreases with increasing temperature, probably due to desolvation of parts of the monomer which make it more hydrophobic.

The effect of pressure on a series of alkyltrimethylammonium bromides and on sodium dodecyl sulfate has been studied. The cmc increases upto pressures of about 1000 atmospheres and decreases with further increase of pressure.[77-79] This is due to water structure destruction by the applied thrust to assist wider distribution of the surfactant molecules in solution to oppose their tendency of association. The decrease in cmc may be due to an increase in the dielectric constant of water, making less electrical work necessary to bring a monomer into a micelle.

Micellar Aggregational Systems

It is generally assumed that micelles at concentrations close to their cmc are roughly spherical.[65,66] The hydrophobic part of the aggregate forms the core of the micelle while the polar head groups are located at the micelle-water interface in contact with and hydrated by a number of water molecules. When the surfactant concentration markedly exceeds the cmc, the shape of the spherical or ellipsoidal micelle undergoes gradual changes. It elongates to assume cylindrical or lamellar structures. If the concentration is further increased, lamellar structures are converted to a hexagonal packing of water cylinders, upon addition of oil and a short chain alcohol, these water cyliners can be converted to a water-in-oil micro-emulsion, for which structures were well established from X-ray diffraction studies.[80,81] By changing the physico-chemical conditions, such as temperature, pH, addition of electrolytes[82,83] etc., it is also possible to

induce structural transition. Fig. 1.3 shows different structures which are formed in the surfactant solution on increasing the concentration of a surfactant.

The number of surfactant molecules which aggregate to form a micelle is called aggregation number (N_{ag}), which determines the size and geometry of the micelle. N_{ag} for surfactants in aqueous solution generally range between 10 and 100. Many techniques, like light scattering,[84-87] viscosity,[88-91] conductivity,[92] ultrasonic absorption,[93] solution calorimetry[94] and small-angle neutron scattering (SANS)[95,96] are employed to detect the structural transition in micellar systems. Micelles that are found close to the cmc are really somewhat smaller than micelles at higher concentrations, at which light scattering measurements are usually carried out. Recent calculations based on geometrical considerations indicated, however, ellipsoidal rather than spherical structures for the most common small micelles.[97,98] Similarly micelles were proposed to be consistent with a spherical shape only if the polar head groups are buried in the micellar core or if there is a cavity in the centre of the micelle,[99] although the validity of the arguments supporting this latter interpretation has been questioned.[100] Results of light scattering, viscosity, diffusion and ultracentrifugation studies on nonionic Cetomacrogol micelles indicated their shape to be ellipsoidal with an axial ratio of 2:1.[101]

Surface active molecules self-assemble as micelles or vesicles in dilute aqueous solutions so as to minimize the contact between their

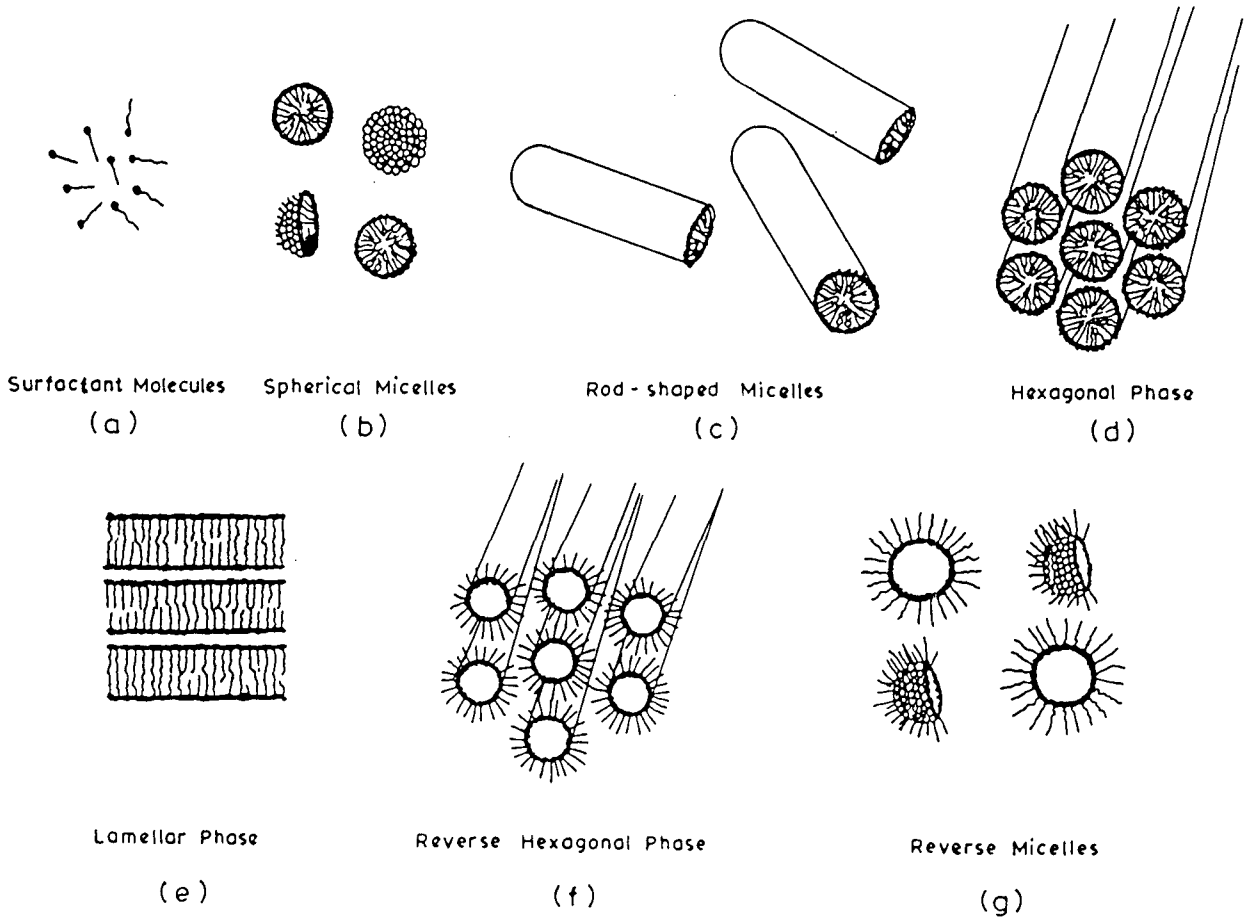


Fig. 1.3: The order of phase structures formed upon increasing surfactant concentration.

hydrophobic tails and water. As a result, the interior of micelles and spherical shells of vesicles are highly non-polar, capable of accommodating other non-polar molecules. Consequently, hydrocarbon molecules which are only sparingly soluble in water can be solubilized in significant amounts in aqueous surfactant solutions.[36,66,102]

Some water molecules may be entrapped by the micelle[103,104] and under certain circumstances part of the hydrocarbon chain may extend into the aqueous phase.[105] Ample evidence exists for extensive hydration of the micellar surface.[106,107] The amount of water in the micellar interior varies from surfactant to surfactant, but water is considered, at present, to penetrate the micellar surface only up to distances of approximately three to six carbon atoms.[108,109] The interior, or core, of the micelle has generally been inferred to be hydrocarbon-like from electron spin resonance (esr)[110] and nuclear magnetic resonance (nmr)[111] spectroscopy and from the utilization of fluorescent probes.[112,113]

Packing considerations constitute a factor which involves the nature of the head and tail groups of the surfactant. Ninham *et al.*[114,115] have devised a critical ratio (R_p) with associated limits for the possible aggregration shapes, given in Fig. 1.4 as:

$$R_p = V_h/A_o l_c \tag{1.7}$$

where V_h is the volume of the amphiphile's hydrocarbon tail, A_o is the cross-sectional area per surfactant molecule, and l_c is the length of the

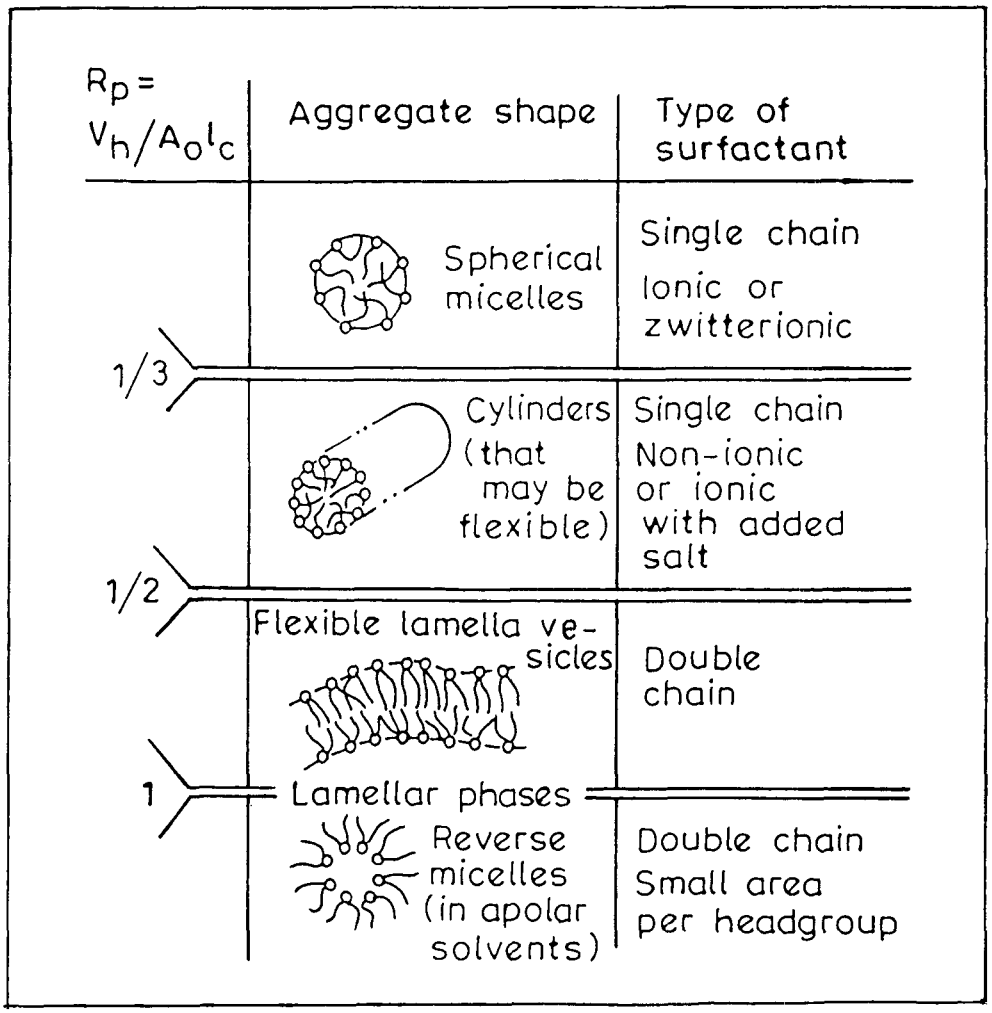


Fig. 1.4: Schematic diagram of possible aggregate shapes according to the packing factor, $R_p = V_h / A_0 l_c$, criterion.

fully extended hydrocarbon tail. The optimum cross-sectional area is determined experimentally by X-ray differaction of bilayer systems, while the volume and length of the hydrocarbon tail may be calculated using following Tanford equations.[97]

$$V = (274 + 26.9n)\ Å^3 \quad (1.8)$$

$$l_c = (1.5 + 1.265n)\ Å \quad (1.9)$$

(n is the number of carbon atoms in the hydrocarbon chain).

Properties

Solubilization **:** An enhanced dissolution of otherwise slightly soluble organics in aqueous solutions is brought about by the presence of surfactant micelles in the system. This is known as solubilization. In other words, the term solubilization implies the formation of a thermodynamically stable isotropic solution of a substrate (the solubilizate), normally insoluble in a given solvent, by the addition of a surfactant (the solubilizer). Indeed, surfactants have been utilized in several fields to enhance the solubility of organic compounds.[116,117] Solubilization is, of course, closely related to micellization. Since little or no solubility increase is observed until the cmc of the surfactant is reached, but once the micelles are fully formed its increase is directly proportional to the concentration of the surfactant over a large range. The observation of solubility changes as a function of surfactant concentration has, in fact,

led to the determination of numerous cmc values. Solubilized systems are generally water-continuous dispersions but may be oil-continuous also.

Solubilization into aqueous media is of major practical importance in such areas as the formulation of products containing water-insoluble ingredients, where it can replace the use of organic solvents or cosolvents; in detergency, where solubilization is believed to be one of the major mechanisms involved in the removal of oily soil; in micellar catalysis of organic reactions; and in emulsion polymerization, where it appears to be an important factor in the initiation step. Solubilization into non-aqueous media is of major importance in dry cleaning. Solubilization by surface-active agents, particularly as it relates to pharmaceutical and biological applications, is the subject of a book by Elworthy *et al.*[66]

If the solubility of a normally solvent-insoluble material is plotted against the concentration of the surfactant solution that is solubilizing it, we find that the solubility is very less at concentrations below the cmc of the surfactant but rises abruptly once the cmc has been reached. This indicates that solubilization is a micellar phenomenon, since it occurs only to a negligible extent at concentrations where micelles, if they exist at all, are found only in insignificant numbers.

***Micellar Catalysis*:** During the past several years there has been intense interest in the catalysis of organic reactions by micelles. This interest was awakened by the discovery of similarities between micelles and cell

membranes and by the use of micelles as models for enzyme-catalyzed reactions.

The effect of micelles on organic reactions can be attributed to both electrostatic and hydrophobic interaction. Electrostatic interaction may affect the rate of a reaction either by its effect on the transition state of the reaction or by its effect on the concentration of reactant in the vicinity of the reaction site. Thus, a cationic micelle with its multiplicity of positively charged hydrophilic heads may catalyze the reaction between a nucleophilic anion and a neutral substrate by delocalizing the negative charge developing in the transition state of this reaction, thereby decreasing the energy of activation of the reaction. It may also catalyze the reaction by increasing the concentration of nucleophilic anion at the micelle-water interface close to the reactive site of the substrate.

Efforts to enhance the effectiveness of micellar catalysis have included investigation of multi-charged[118] and functional micelle forming surfactants[119-123] as well as attachment to macromolecular backbones.[124,125]

Micellar catalysis in non-polar media involves the solubilization of reactants (polar) in the hydrophilic portions of reversed micelles.[126] Properties of the polar cavity may be strongly affected by small amounts of water which is expected to behave very differently from ordinary water because of extensive binding and orientation effects.

One of the most spectacular catalytic effects observed so far has been for the aquation of the tris (oxalato) chromium(III) anion.[127,128] The aquation is up to 5.4 million times faster in non-polar medium containing octadecyltrimethyl ammonium tetradecanoate than in bulk water.

Early studies have shown that both electrostatic and geometric factors may be important in micellar catalysis. The kinetics of alkaline decomposition of indoaniline dyes catalysed in SDS and TX-100 micelle can be used to determine the surfactant cmc, the partition coefficient of the dye in the micelles, and the rate constant for decomposition.[129]

Micellar solublization has also been shown to retard alkaline decomposition when the reactive site is buried within the micellar interior where hydroxide attack is significantly activated.

The effects of surfactants, at concentrations both below and above their cmc, on enzyme catalyzed reactions have been studied in order to gain a better understanding of the mechanisms and the active sites involved in enzyme catalysis. In an investigation, the influence of the nonionic surfactant polyoxyethylene (9.5) diisobutylphenol (Polysorbate 80) on the Mylase P (a mixture of enzymes with high arylsulfatase activity) catalyzed hydrolysis of arylsulfate esters has been studied.[130] The rate constant for the nonenzymatic acid-catalyzed hydrolysis of potassium 2,4-dichloronaphthyl sulfate is considerably enhanced by Polysorbate 80. The enzymatic hydrolysis of the same substrate, however, is considerably retarded by micellar Polysorbate 80.[130]

Naturally occurring micelle forming systems, such as phospholipids and bile salts (e.g., cholic and desoxycholic acids) as well as synthetic surfactants affect the rates of numerous chemical reactions *in vivo* and *in vitro*.[131-133] The effects of micellization on enzymatic reactions and other biochemical processes have also been studied.[134,135]

In short, micellar media have been extensively used to affect rates of numerous organic and inorganic reactions. There are many reviews available on this subject.[126,136-140] The first studies of micellar effects upon chemical reactions involved equilibria. Ionic micelles were found to have striking effects upon the protonation of indicator dyes.[141,142]

The study of the kinetic effects of micellar solubilization began at a later date.[143] The catalysis or inhibition of solubilized species involve many kinds of interactions and may vary with the nature of the surfactant.

C. Pseudophase Model of Micellar Catalysis

Micellar effects upon reaction rates and equilibria have generally been discussed in terms of the pseudophase model. The model aids in the interpretation of the catalytic activity of functionalized micelles used as models for enzymatic sites[144] and is applicable to the effects of reverse micelles, microemulsions, and vesicles on reaction rates and equilibria. Bunton – "father of micellar kinetics"[145] – has observed[146]: "The development of a quantitative understanding of chemical reactivity in solution has depended on the willingness of chemists to use models that

are no more than crude approximations. For this reason it is useful to accept the pseudophase model, despite its imperfections, until it either fails to fit the data, or is replaced by a better model".

Micellar catalysis in water is premised in terms of reaction occurring either in the micellar or aqueous pseudophase.[43,47] This model was for the first time applied to the inhibition of ester saponification by anionic micelles[148], and afterwards to catalysis of the spontaneous hydrolysis of dinitrophenyl phosphates[149,150] and sulphates[151] by cationic micelles. The pseudophase description of micellar catalysis and inhibition assumes that the relation between overall reaction rate and surfactant concentration, for a given total concentration of reactants, can be explained in terms of the concentrations of each reactant in water and in the micelles, and the rate constants in the aqueous and micellar pseudophases.

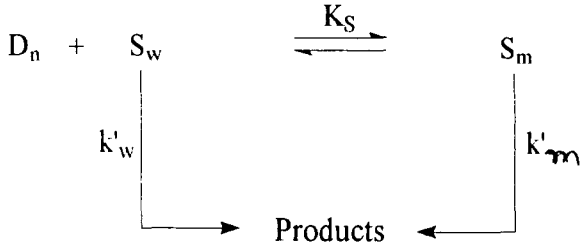
Provided that equilibrium is maintained between the aqueous and micellar pseudophases (designated by subscripts w and m) the overall reaction rate will be the sum of rates in water and the micelles and will therefore depend upon the distribution of reactants between each pseudophase and the appropriate rate constants in the two pseudophases. Early studies of reactivity in aqueous micelles showed the importance of substrate hydrophobicity in determining the extent of substrate binding to micelles; for example, reactions of a very hydrophilic substrate could be

essentially unaffected by added surfactant, whereas large effects were observed with chemically similar, but hydrophobic substrates.[23,138,148]

Ester saponification was a favoured reaction for this type of study, because the hydrophobicity of the acyl moiety could easily be controlled by increasing the length of an *n*-alkyl group, and saponification of *p*-nitrophenyl *n*-alkanoates could be followed with very dilute substrate. Substrate concentration is an important factor, because provided that it is kept low it is reasonable to assume that the micelle structure is relatively unperturbed.

Menger and Portnoy[148] developed a quantitative treatment which adequately described inhibition of ester saponification by anionic micelles. Micelles bound hydrophobic esters, and anionic micelles excluded hydroxide ion, and so inhibited the reaction, whereas cationic micelles speeded saponification by attracting hydroxide ion.[152]

Provided that only substrate distribution has to be considered, which is the situation for micelle-inhibited bimolecular or spontaneous unimolecular, reactions, Scheme 1.7 describes substrate distribution and reaction in each pseudophase.[149]



Scheme 1.7

In Scheme 1.7, D_n denotes micellized surfactant, S is substrate, and k'_w and k'_m are first-order rate constants. The binding constant, K_S, is written in terms of the molarity of micellized surfactant, but it could equally be written in terms of the molarity of micelles. The two constants differ in magnitude by the aggregation number of the micelles.

The concentration of micellized surfactant is that of total surfactant less than that of monomer which is assumed to be given by the critical micelle concentration (cmc). The overall first-order rate constant, k_ψ, is then given by Eq. (1.10).

$$k_\psi = \frac{k'_w + k'_m K_S([D] - cmc)}{1 + K_S([D] - cmc)} \qquad (1.10)$$

This equation is formally similar to the Michaelis-Menten equation of enzyme kinetics, although the analogy is limited because most enzymic reactions are studied with substrate in large excess over enzyme. Equation (1.10) could be rearranged to give (1.11) which is formally similar to the Lineweaver-Burk equation, and which permits calculation of k'_m and K_S, provided that k'_w is known.[148,152]

$$\frac{1}{(k'_w - k_\psi)} = \frac{1}{(k'_w - k'_m)} + \frac{1}{(k'_w - k'_m) K_S[D_n]} \qquad (1.11)$$

Equations (1.10) and (1.11) depend on some major assumptions, in particular that the cmc gives the concentration of monomeric surfactant

and that rate and binding constants in the micellar pseudophase are unaffected by reactants and products. These equations have been used very extensively and provided the basis for quantitative analysis of micellar rate effects.

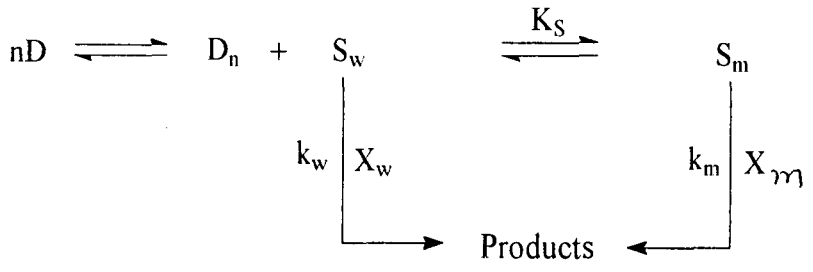
Equation (1.10) is generally used to estimate the rate constant, k'_m, in the micellar pseudophase, but for inhibited bimolecular reactions it provides an indirect method for estimation of otherwise inaccessible rate constants in water. Oxidation of a ferrocene to the corresponding ferricinium ion by Fe(III) is speeded by anionic micelles of SDS and inhibited by cationic micelles of cetyltrimethylammonium bromide or nitrate.[153] Oxidation of ferrocene by ferricyanide ion in water is too fast to be easily followed kinetically, but the reaction is strongly inhibited by anionic micelles of SDS which bind ferrocene, but exclude ferricyanide ion. Thus reaction occurs essentially quantitatively in the aqueous pseudophase, and the overall rate depends upon the rate constant in water and the distribution of ferrocene between water and the micelles. It is easy, therefore, to calculate the rate constant in water from this micellar inhibition.

Equations (1.10) and (1.11) generally fail for bimolecular, micelle-assisted reactions. Equation (1.10) predicts that the first-order rate coefficients should reach a constant, limiting value at high surfactant concentration when the substrate is fully micellar bound, but rate maxima are observed for the corresponding nonsolvolytic bimolecular reactions.

The rate ¾ surfactant concentration profiles can be treated quantitatively by taking into account the distribution of both reactants between water and micelles. This can be done by extending (1.10), and a simple formalism involves writing the first-order rate constants k'_w and k'_m in terms of second-order rate constants in water and micelles, and reactant concentrations in each pseudophase.[144,147,154-156] However, one immediately runs into the problem of defining concentration in the micellar pseudophase. One approach is to write concentration in terms of moles of reagent per dm^3 of micelles, or to assume some volume of the micellar pseudophase, V_m, in which reaction takes place. The problem is similar to that of comparing second-order rate constants, written conventionally in the dimensions mol^{-1} dm^3 s^{-1}, for a variety of solvents. The comparisons are completely different if concentrations are written in terms of molarities or mole fractions.

Another approach is to define concentration in the micellar pseudophase in terms of mole ratio. Concentration is then defined unambiguously, and the equations take a simple form.[157-161]

Scheme 1.8 shows reaction between the substrate S and nucleophile



Scheme 1.8

X (or any second reactant). The second reactant is generally in large excess over the substrate establishing pseudo-first-order conditions, so that

$$k'_w = k_w [X_w] \tag{1.12}$$

$$k'_m = k_m m_X^S \tag{1.13}$$

where k_w and k_m are second-order rate constants for reaction in aqueous and micellar pseudophases, respectively, and m_X^S is the mole ratio of micellar bound reactive nucleophile to micellized surfactant given by

$$m_X^S = [X_m]/[D_n] \tag{1.14}$$

Substituting Eqs. (1.12) and (1.13) in equation (1.10), we get

$$k_\psi = \frac{k_w [X_w] + k_m K_S m_X^S [D_n]}{1+K_S [D_n]} \tag{1.15}$$

$$= \frac{k_w [X_w] + k_m K_S [X_m]}{1+K_S [D_n]} \tag{1.16}$$

These, or similar, equations readily explain why first-order rate constants of micelle-assisted bimolecular reactions typically go through maxima with increasing surfactant concentration if the overall reactant concentration is kept constant. Addition of surfactant leads to binding of both reactants to micelles, and this increased concentration increases the reaction rate. Eventually, however, increase in surfactant concentration

dilutes the reactants in the micellar pseudophase and the rate falls. This behaviour supports the original assumption that substrate in one micelle does not react with reactant in another, and that equilibrium is maintained between aqueous and micellar pseudophases.

Equation (1.15) or (1.16) and others, which are essentially identical but are written in different ways, can be applied to bimolecular micelle-assisted reactions provided that the distribution of both reactants can be determined.

The final form of the kinetic equation (1.15) will depend upon the properties of the second reactant: whether it is a neutral molecule, a hydrophilic or hydrophobic coion, a counterion to the micelle, or in the complex systems, an anion of a weak organic acid XH.

D. Statement of the Problem

Ninhydrin is widely used in biochemical and medical settings for the analysis of amino acids and also used as a latent fingerprint reagent.[162,163] The analysis is based on the formation of purple-coloured diketohydrindylidenediketohydrindamine (DYDA), also known as Ruhemann's purple.[2,3] The chemistry of this reaction has, therefore, been intensively studied at many occasions.[6,10,164] This reaction has biological importance too as it forms a model for several biochemical reactions that occur in the metabolism of deamination and transpeptidation.[165,166]

Micelles are known to affect the rate of reactions by differential distribution of the substrates inside and outside the micelles and by changing the thermodynamic parameters of the reaction.[23,138,139,167] The explanation of the mechanism of catalysis of reaction rates has attracted the attention of many researchers in view of the analogies drawn between the micellar and enzyme catalyses.

The above facts delineate the effective role of ninhydrin-amino acid reactions and the scope of a micelle in the course of such reactions. This method has a potential to enhance contrast and visualisation of ninhydrin developed finger prints and is bound to improve the existing available methods in forensic science. Due to its importance and being a typical addition-elimination type reaction, systematic kinetic and mechanistic studies of the formation of DYDA between DL-alanine, DL-methionine, DL-threonine, L-tyrosine, L-glutamic acid and L-arginine and ninhydrin were performed under different experimental conditions. Also, no report is found in the ninhydrin literature for the rate and absorbance enhancements of the Ruhemann's purple formation in presence of organic solvents. The present work is, therefore, concerned with the results obtained in the absence and presence of CTAB micelles and in various non aqueous organic solvents.

CHAPTER - 2

EXPERIMENTAL

Materials

The chemicals used throughout the whole study are listed in Table 2.1. Cetyltrimethylammonium bromide (CTAB) was used as received. However, its purity was ascertained by the absence of minimum in surface tension *vs.* log [CTAB] plot. Rest of the chemicals given in Table 2.1 were of reagent grade and used as received.

Preparation of Solutions

Double-distilled and deionized water was used to prepare solutions. The specific conductivity of this water was in the range (1-2) x 10^{-6} S cm^{-1}.

(i) *Buffer solutions*

Sodium acetate-acetic acid buffers were prepared by mixing appropriate volumes of 0.20 mol dm^{-3} acetic acid and 0.20 mol dm^{-3} sodium acetate solutions.[168]

(ii) *CTAB solution*

CTAB solution was prepared by dissolving appropriate amount of CTAB in the buffer solution of required pH.

(iii) *Ninhydrin and amino acid solutions*

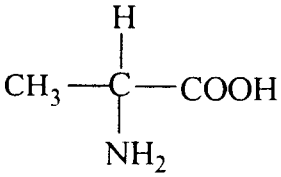
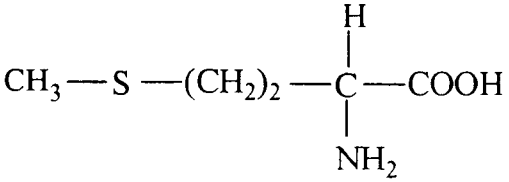
Stock solutions of ninhydrin and amino acids were also prepared in buffer solutions. The ninhydrin stock solution was stored in a dark bottle.

pH-Measurements

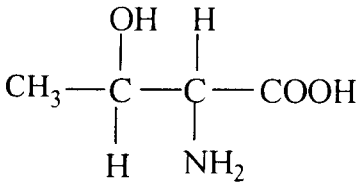
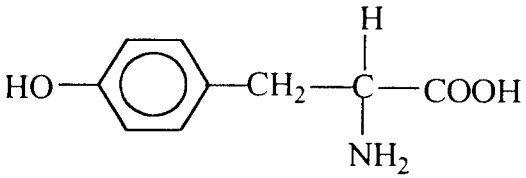
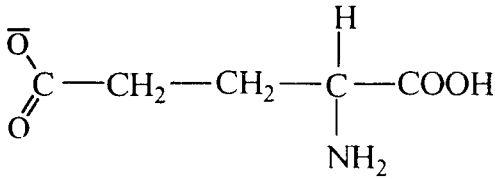
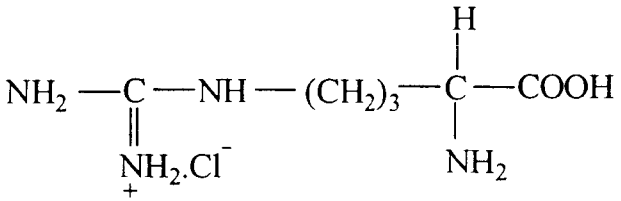
An ELICO pH-meter (Model LI-120, Hyderabad, India) was used for pH measurements. The electrode used was an ELICO CH-41 glass and

TABLE 2.1

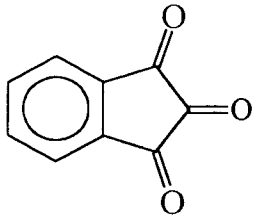
Names and structural formulae of the chemicals used.

Name	Abbreviation	Structure/Formula	Make	% Purity
Surfactant				
Cetyltrimethylammonium bromide	CTAB	$CH_3(CH_2)_{15}\overset{+}{N}(CH_3)_3Br^-$	BDH (England)	99
			Koch-Light (England)	99
Reactants				
(a) DL-Alanine	Ala		SRL (India)	99
(b) DL-Methionine	Met		LOBA (India)	99

contd.

(c) DL-Threonine	Thr		BDH (England)	99
(d) L-Tyrosine	Tyr		s.d. fine (India)	98.5
(e) L-Glutamic acid	Glu		SRL (India)	99
(f) L-Arginine monohydrochloride	Arg		LOBA (India)	99

contd.

(g) Ninhydrin			Merck (India)	99

Solvents

(a) Acetonitrile	AN	CH_3CN	Qualigens (India)	99
(b) 1-Propanol	C_3OH	$CH_3CH_2CH_2OH$	BDH (England)	99.5
			Merck (India)	99
(c) Methyl cellosolve	MCS	$CH_2—OH$ | $CH_2—O—CH_3$	SRL (India)	99
			Merck (India)	99
(d) Dimethyl sulfoxide	DMSO	$CH_3—\overset{\overset{O}{\|\|}}{S}—CH_3$	Merck (India)	99

contd.

Salt				
Sodium acetate	–	CH_3COONa	Merck (India)	99
Acid				
Acetic acid	–	CH_3COOH	Qualigens (India)	99.5
			s.d. fine (India)	99.5

calomel combination electrode. The electrode was stored in pH 7 buffer and was washed in deionized double-distilled water before use. It was then rinsed with pH 7 buffer and the pH-meter was standardized using pH 4 buffer solution. Whenever the solution was changed, the electrode was rinsed with double-distilled water and the surplus water was removed and the pH-meter was restandardized using the pH 4 buffer solution. All pH measurements were made at least in triplicate and they agreed within ±0.02.

Spectra of the Reaction Product

The reaction of ninhydrin with the amino acids used forms a purple-coloured product. This product has two absorption maxima in the visible range[12-20, 169] : λ_{max} = 400, 570 nm. Spectra of the purple-coloured product were recorded in the absence and presence of surfactant micelles (CTAB) and various percentages of organic solvents, viz. acetonitrile (AN), dimethyl sulfoxide (DMSO), methyl cellosolve (MCS) and 1-propanol (C_3OH). The absorption maxima remained in the same position in all the above cases. This indicates that the reaction product of amino acids with ninhydrin is the same in presence of CTAB and organic solvents as in aqueous solutions. The absorption spectra were recorded by Bausch and Lomb Spectronic-20 spectrophotometer between 350 and 600 nm which are shown in Figs. 2.1-2.11.

The results indicate that the ninhydrin-amino acid reactions are catalysed by CTAB micelles.

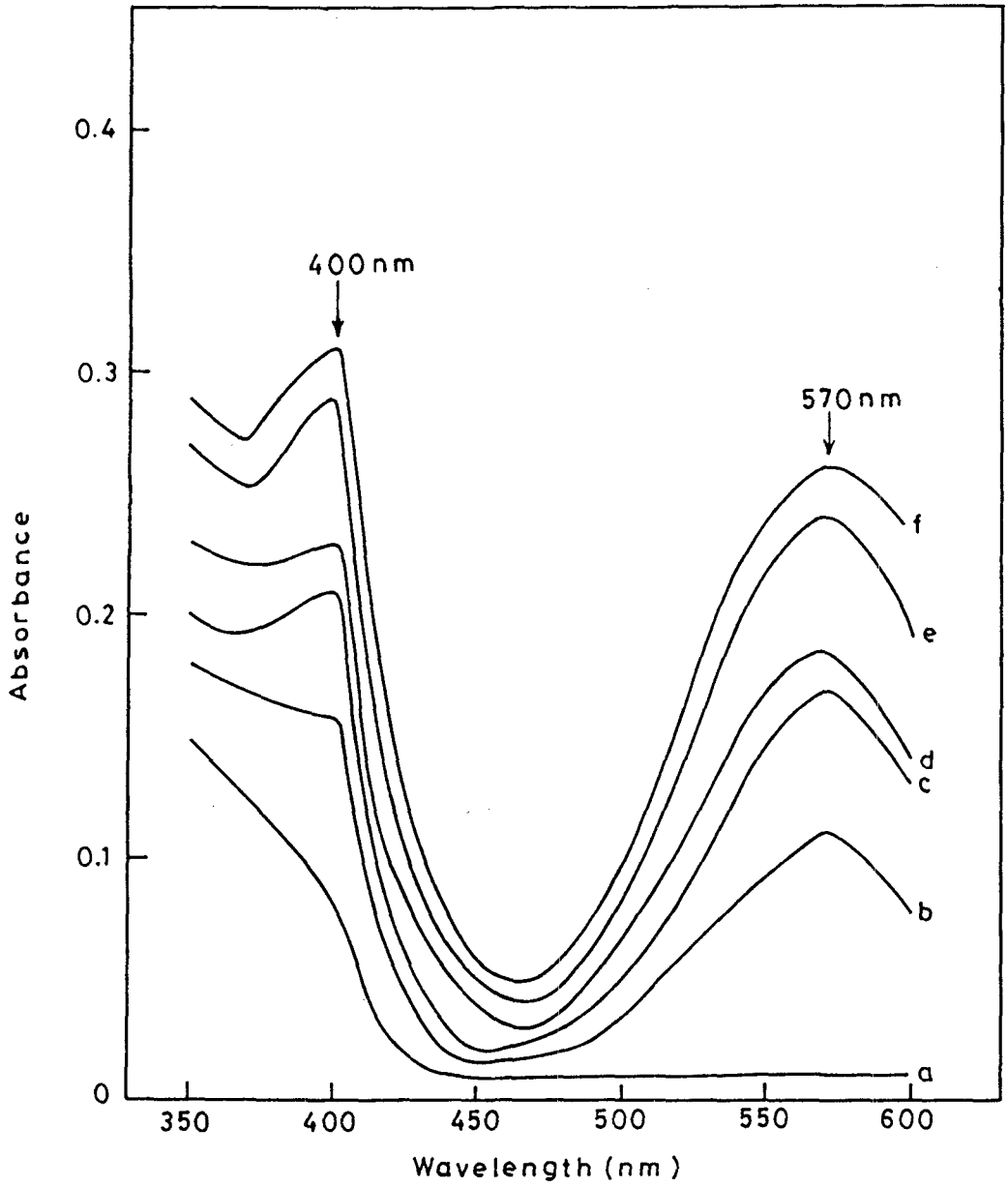


Fig. 2.1: Absorption spectra of the reaction product of alanine with ninhydrin in absence of solvent (a,b), 10% methyl cellosolve (c), 10% 1-propanol (d), 10% dimethyl sulfoxide (e), 10% acetonitrile (f). *Reaction conditions*: $[alanine]_T$ = 1.0 x 10^{-4} mol dm^{-3} (a), 3.0 x 10^{-4} mol dm^{-3} (b–f), $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

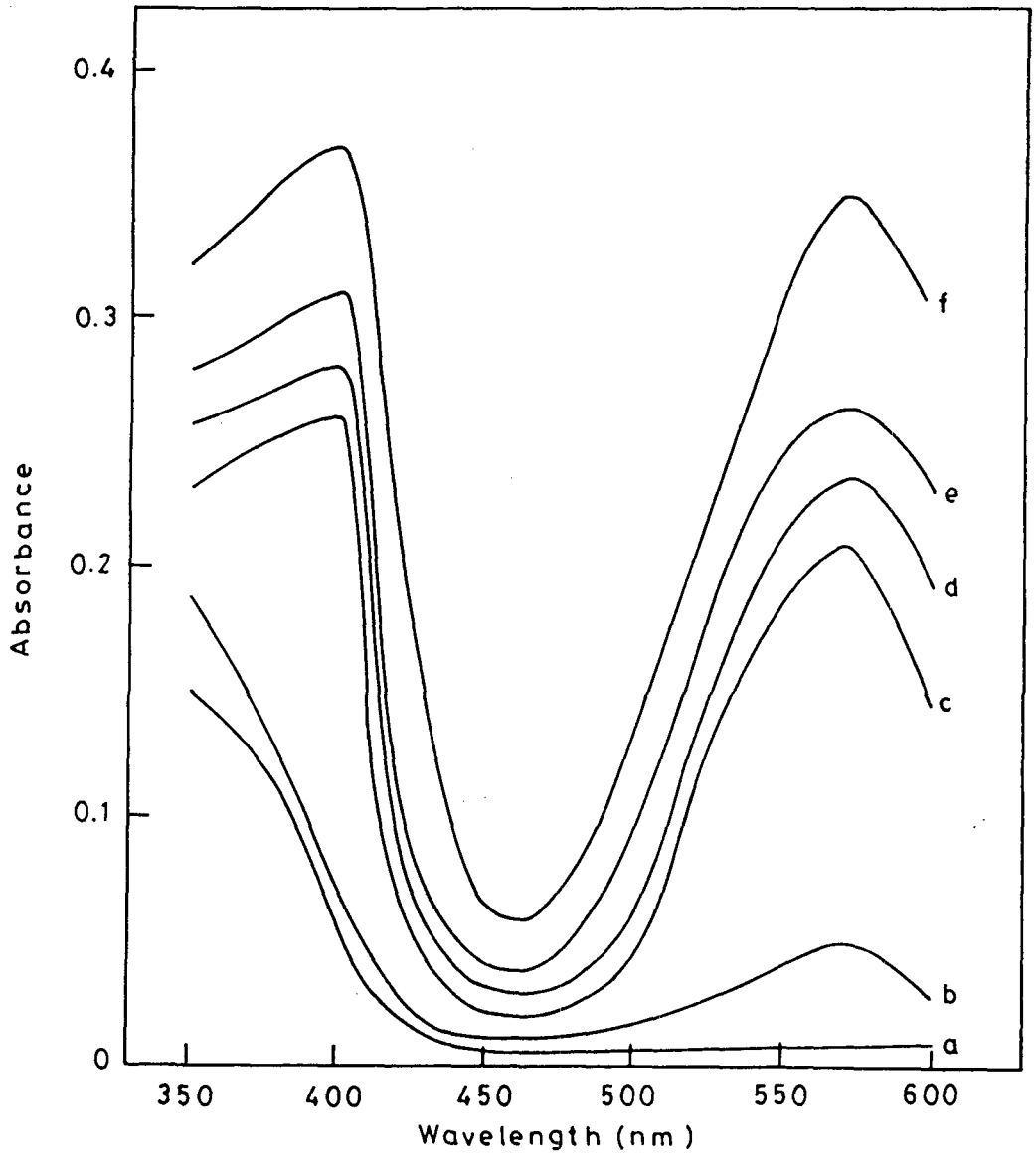


Fig. 2.2: Absorption spectra of the reaction product of methionine with ninhydrin in absence of solvent (a,b), 10% 1-propanol (c), 10% dimethyl sulfoxide (d), 10% methyl cellosolve (e), 10% acetonitrile (f). *Reaction conditions* : $[methionine]_T$ = 1.0 x 10^{-4} mol dm^{-3} (a), 3.0 x 10^{-4} mol dm^{-3} (b–f), $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

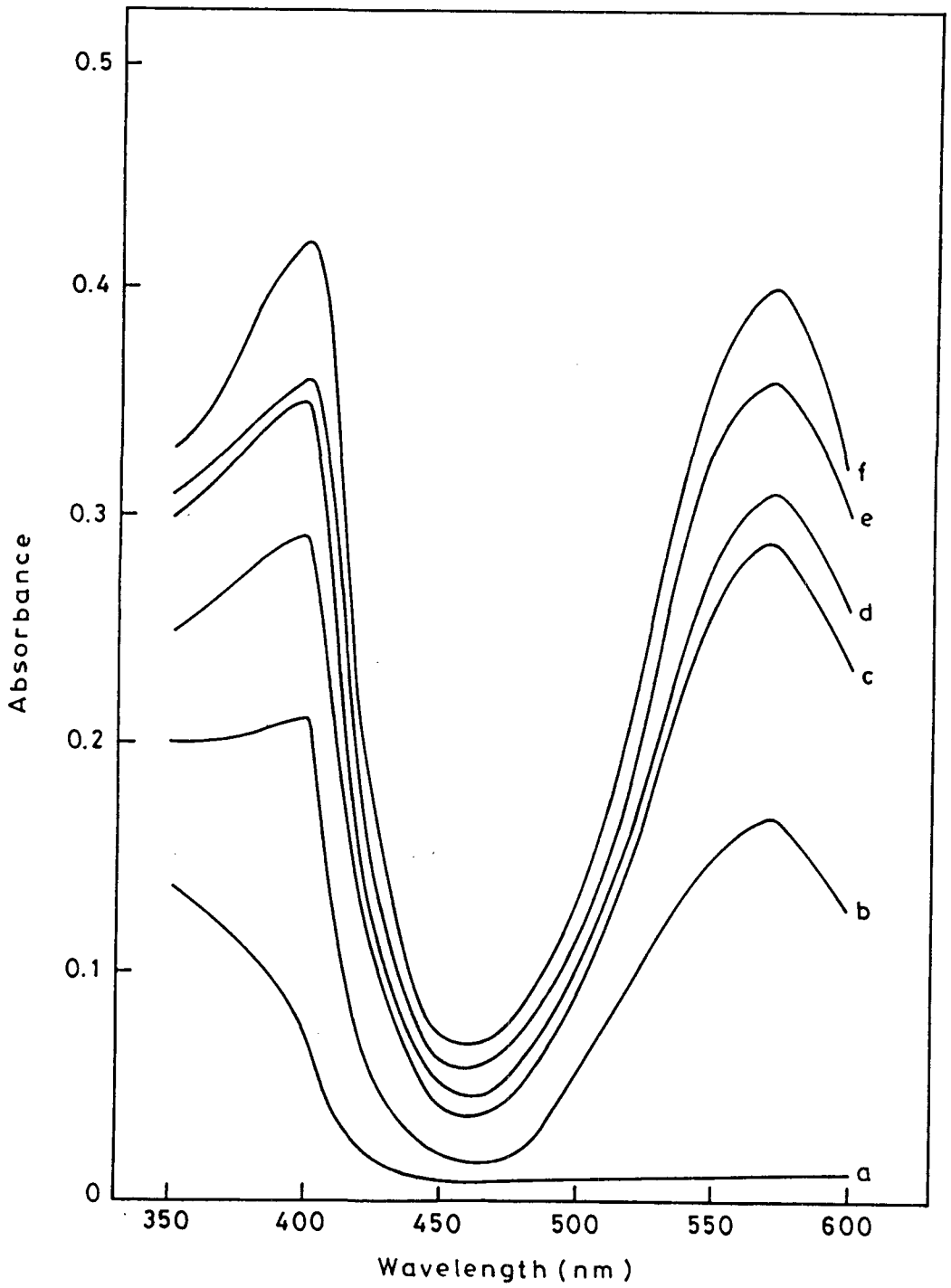


Fig. 2.3: Absorption spectra of the reaction product of threonine with ninhydrin in absence of solvent (a,b), 10% dimethyl sulfoxide (c), 10% methyl cellosolve (d), 10% 1-propanol (e), 10% acetonitrile (f). *Reaction conditions* : $[threonine]_T$ = 1.0 x 10^{-4} mol dm^{-3} (a), 2.0 x 10^{-4} mol dm^{-3} (b–f), $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

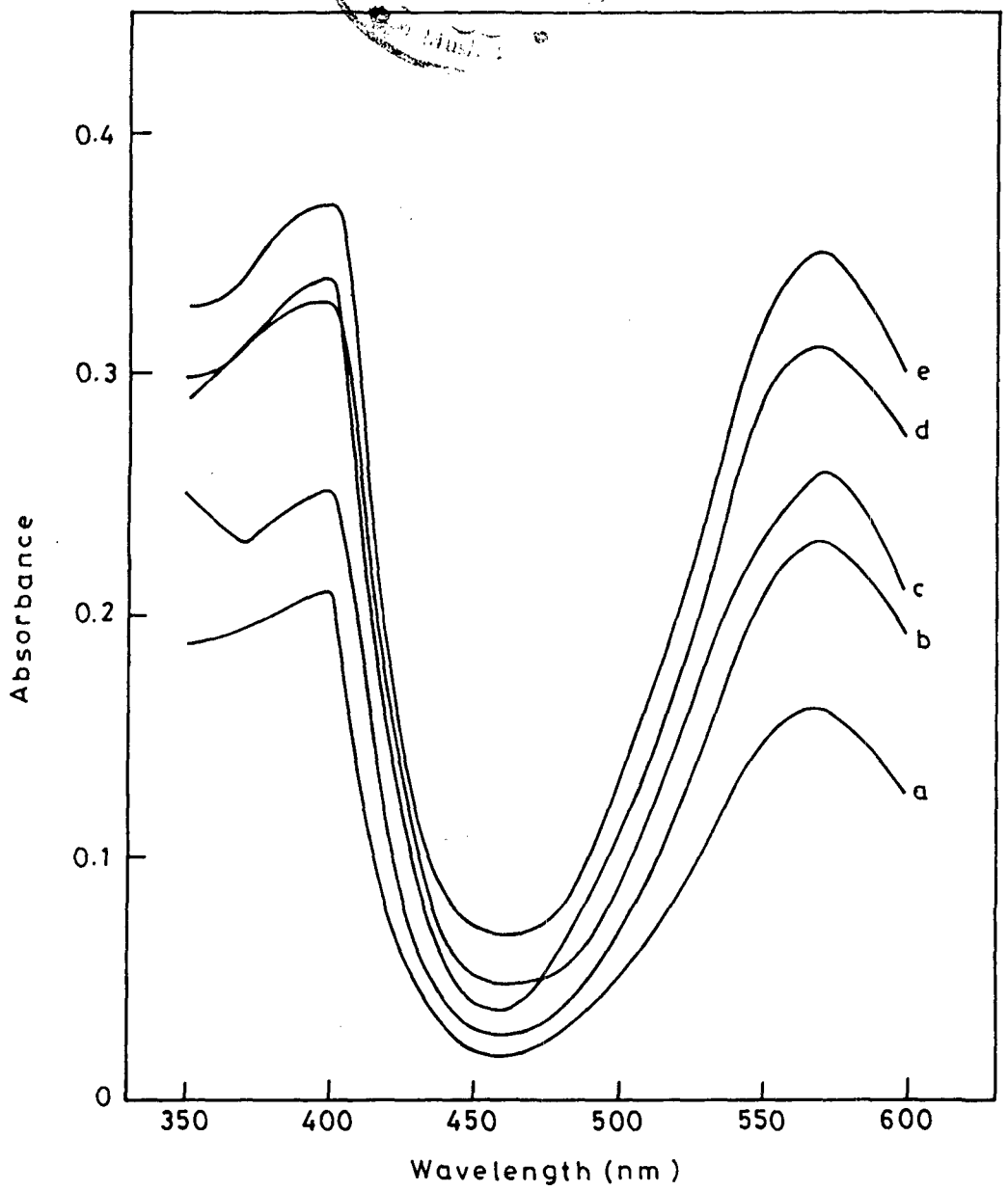


Fig. 2.4: Absorption spectra of the reaction product of tyrosine with ninhydrin in absence of solvent (a), 10% dimethyl sulfoxide (b), 10% 1-propanol (c), 10% methyl cellosolve (d), 10% acetonitrile (e). *Reaction conditions* : $[tyrosine]_T = 1.0 \times 10^{-4}$ mol dm^{-3}, $[ninhydrin]_T = 5.0 \times 10^{-3}$ mol dm^{-3}, pH = 5.0, temp. = 80 °C.

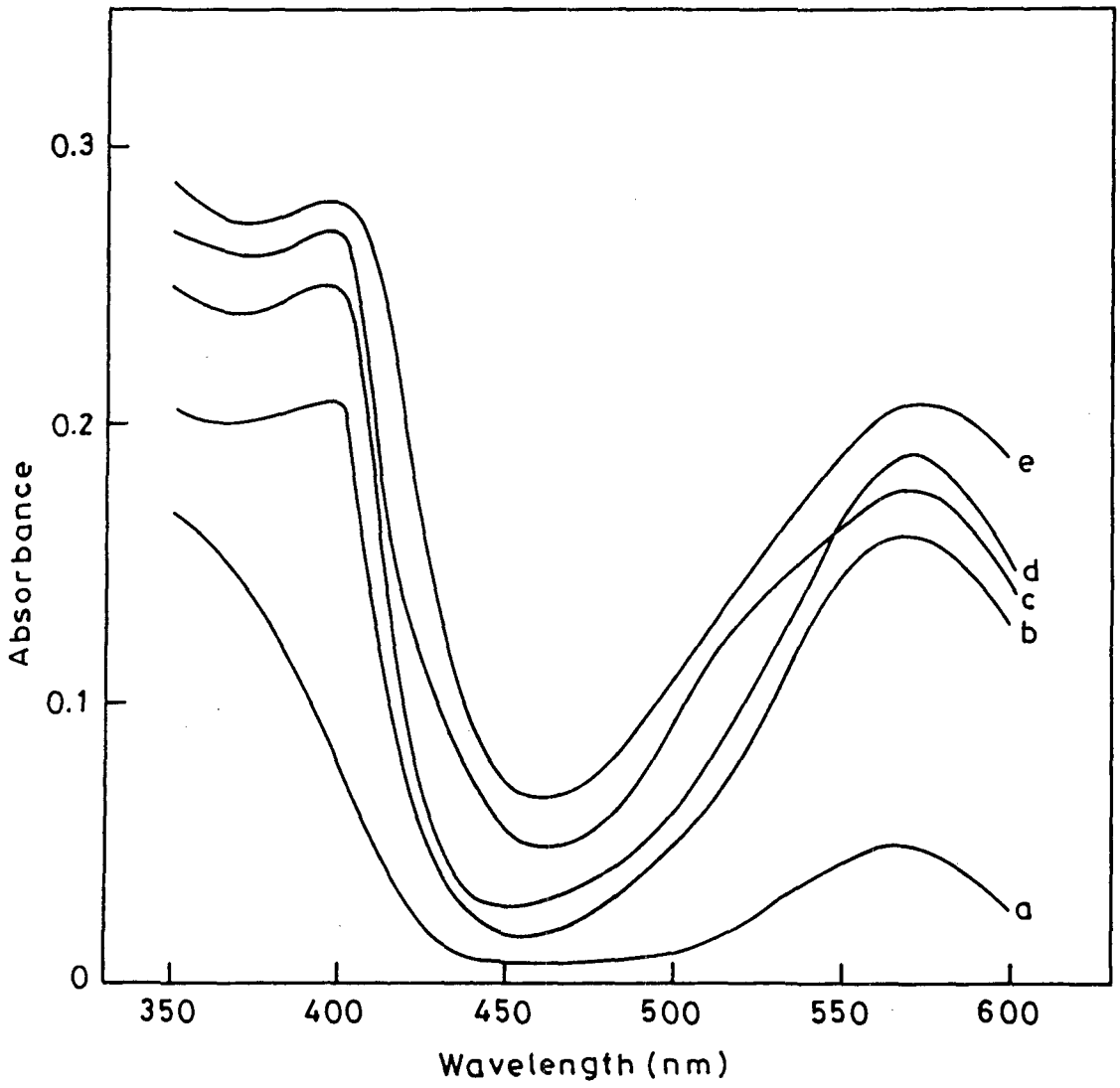


Fig. 2.5: Absorption spectra of the reaction product of glutamic acid with ninhydrin in absence of solvent (a), 10% dimethyl sulfoxide (b), 10% acetonitrile (c), 10% methyl cellosolve (d), 10% 1-propanol (e). *Reaction conditions* : $[\text{glutamic acid}]_T = 1.0 \times 10^{-4}$ mol dm^{-3}, $[\text{ninhydrin}]_T = 5.0 \times 10^{-3}$ mol dm^{-3}, pH = 5.0, temp. = 80 °C.

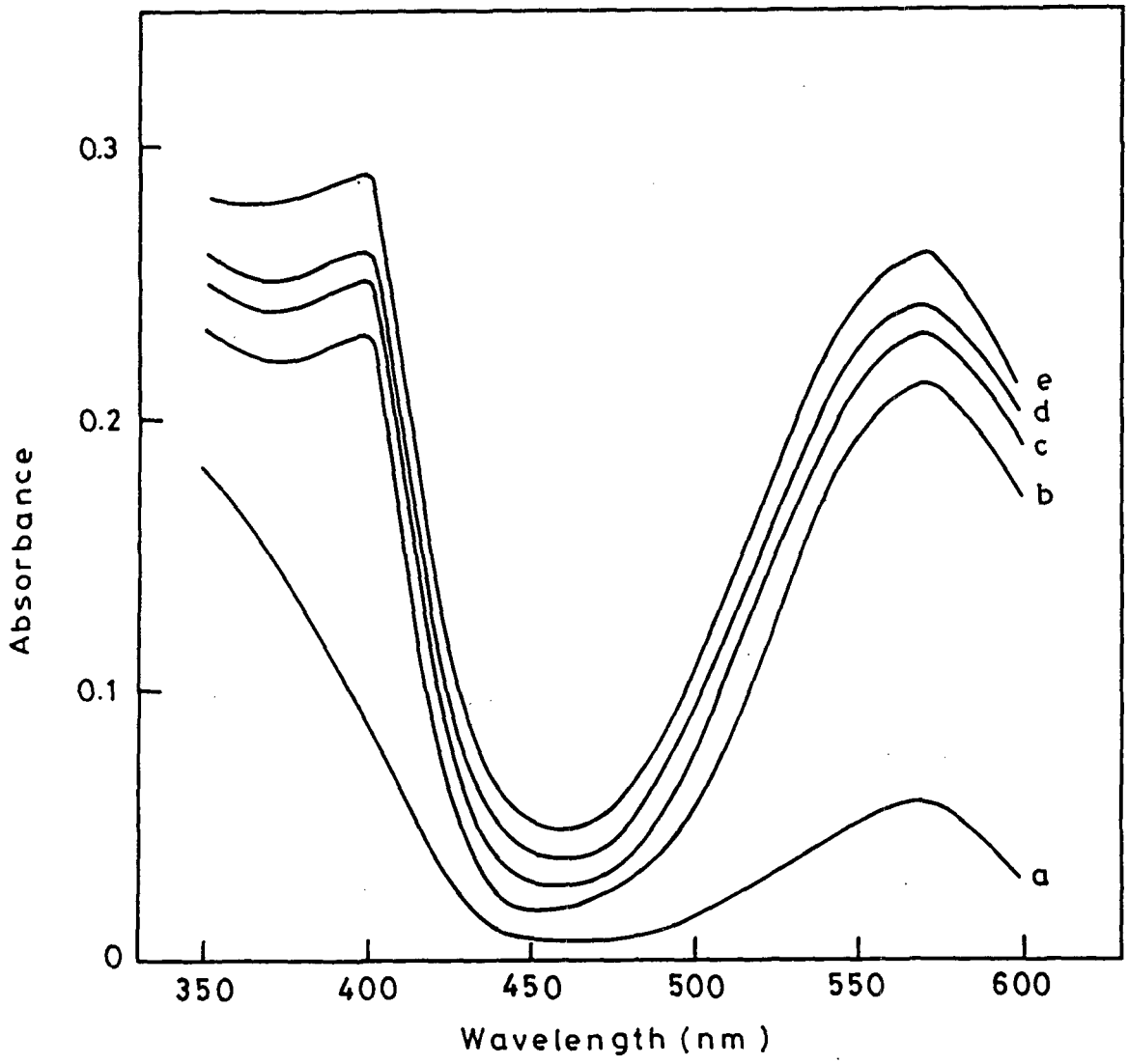


Fig. 2.6: Absorption spectra of the reaction product of arginine with ninhydrin in absence of solvent (a),10% 1-propanol (b), 10% dimethyl sulfoxide (c), 10% acetonitrile (d), 10% methyl cellosolve (e) . *Reaction conditions* : $[arginine]_T$ = 1.0 x 10^{-4} mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

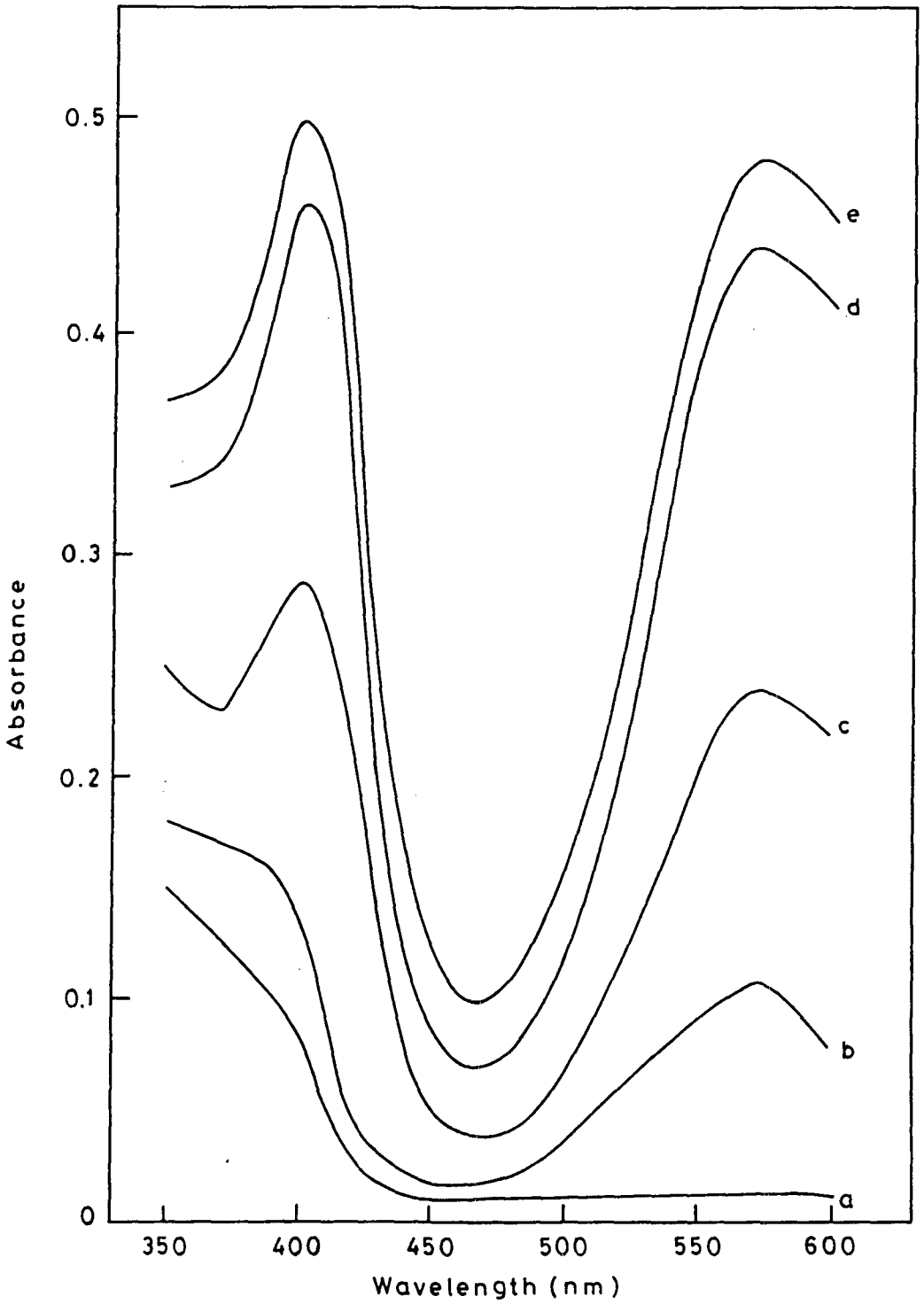


Fig. 2.7: Absorption spectra of the reaction product of alanine with ninhydrin in absence of CTAB (a,b), 5.0 x 10^{-3} mol dm^{-3} CTAB (c), 10.0 x 10^{-3} mol dm^{-3} CTAB (d), 10.0 x 10^{-3} mol dm^{-3} CTAB + 10% DMSO (e). *Reaction conditions* : $[alanine]_T$ = 1.0 x 10^{-4} mol dm^{-3} (a), 3.0 x 10^{-4} mol dm^{-3} (b–e), $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

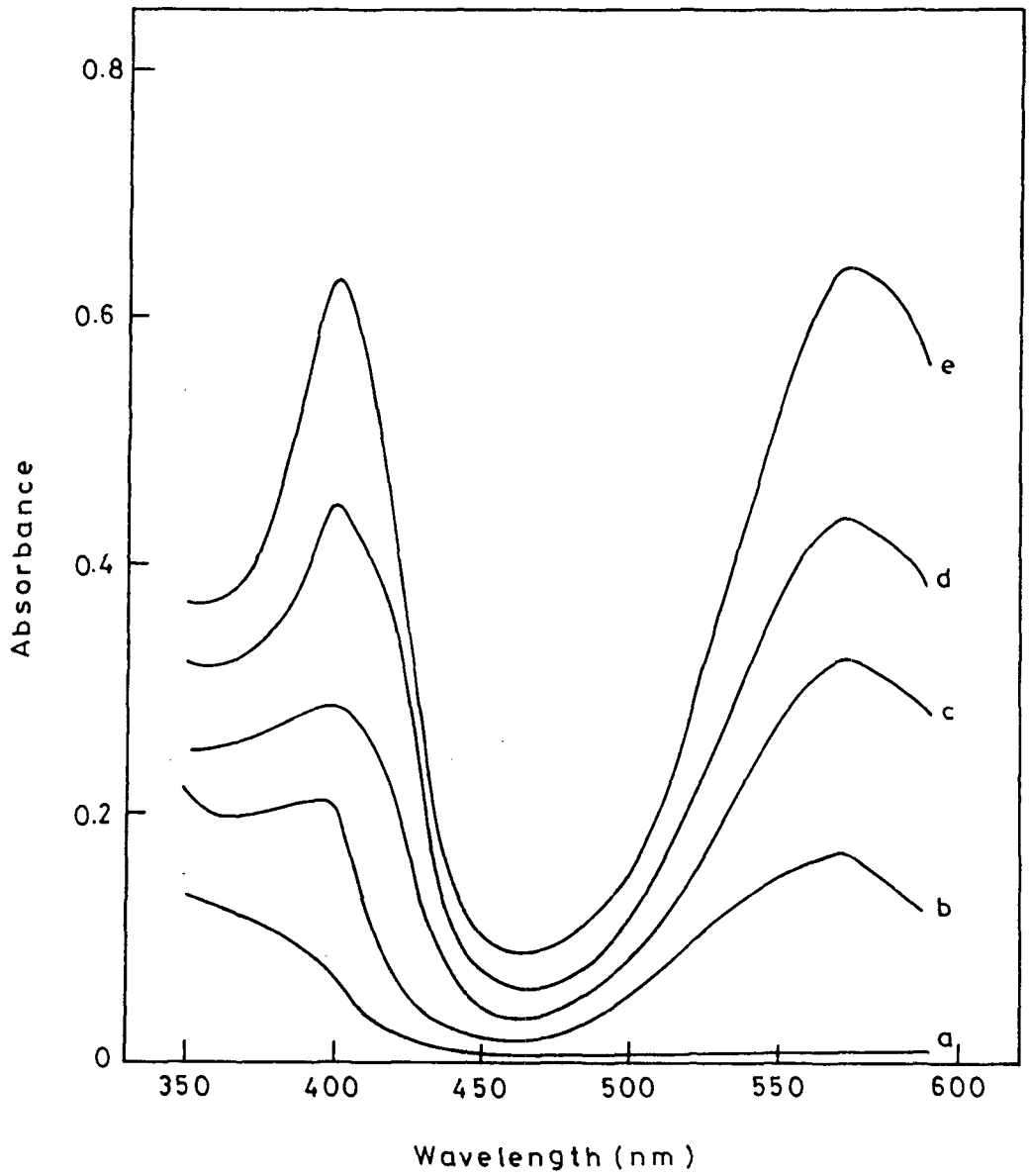


Fig. 2.8: Absorption spectra of the reaction product of threonine with ninhydrin in absence of CTAB (a,b), 5.0 x 10^{-3} mol dm^{-3} CTAB (c), 10.0 x 10^{-3} mol dm^{-3} CTAB (d), 10.0 x 10^{-3} mol dm^{-3} CTAB + 10% DMSO (e). *Reaction conditions* : $[threonine]_T$ = 1.0 x 10^{-4} mol dm^{-3} (a), 2.0 x 10^{-4} mol dm^{-3} (b–e), $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

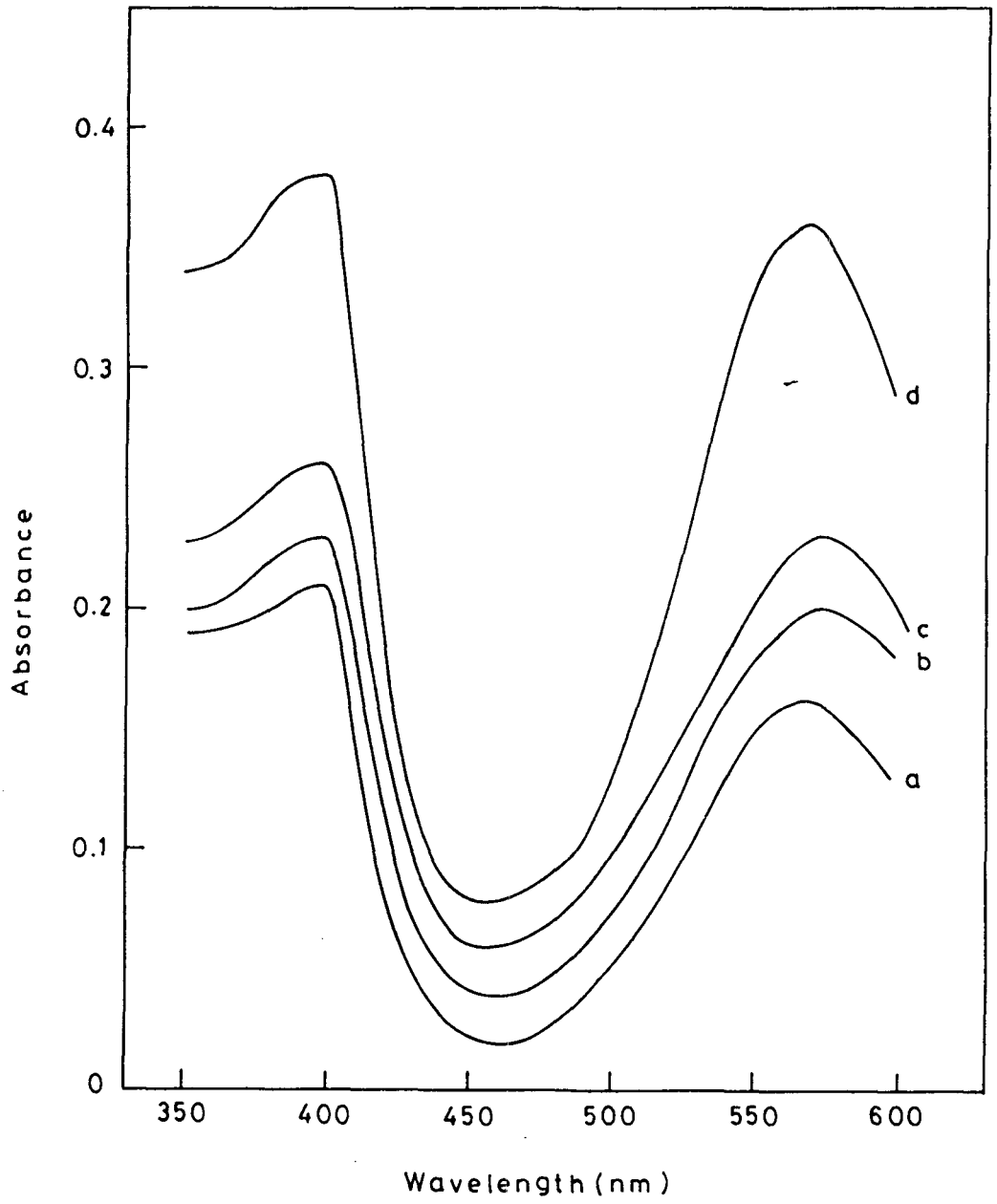


Fig. 2.9: Absorption spectra of the reaction product of tyrosine with ninhydrin in absence of CTAB (a), 5.0 x 10^{-3} mol dm^{-3} CTAB (b), 10.0 x 10^{-3} mol dm^{-3} CTAB (c), 10.0 x 10^{-3} mol dm^{-3} CTAB + 10% DMSO (d). *Reaction conditions* : $[tyrosine]_T$ = 1.0 x 10^{-4} mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

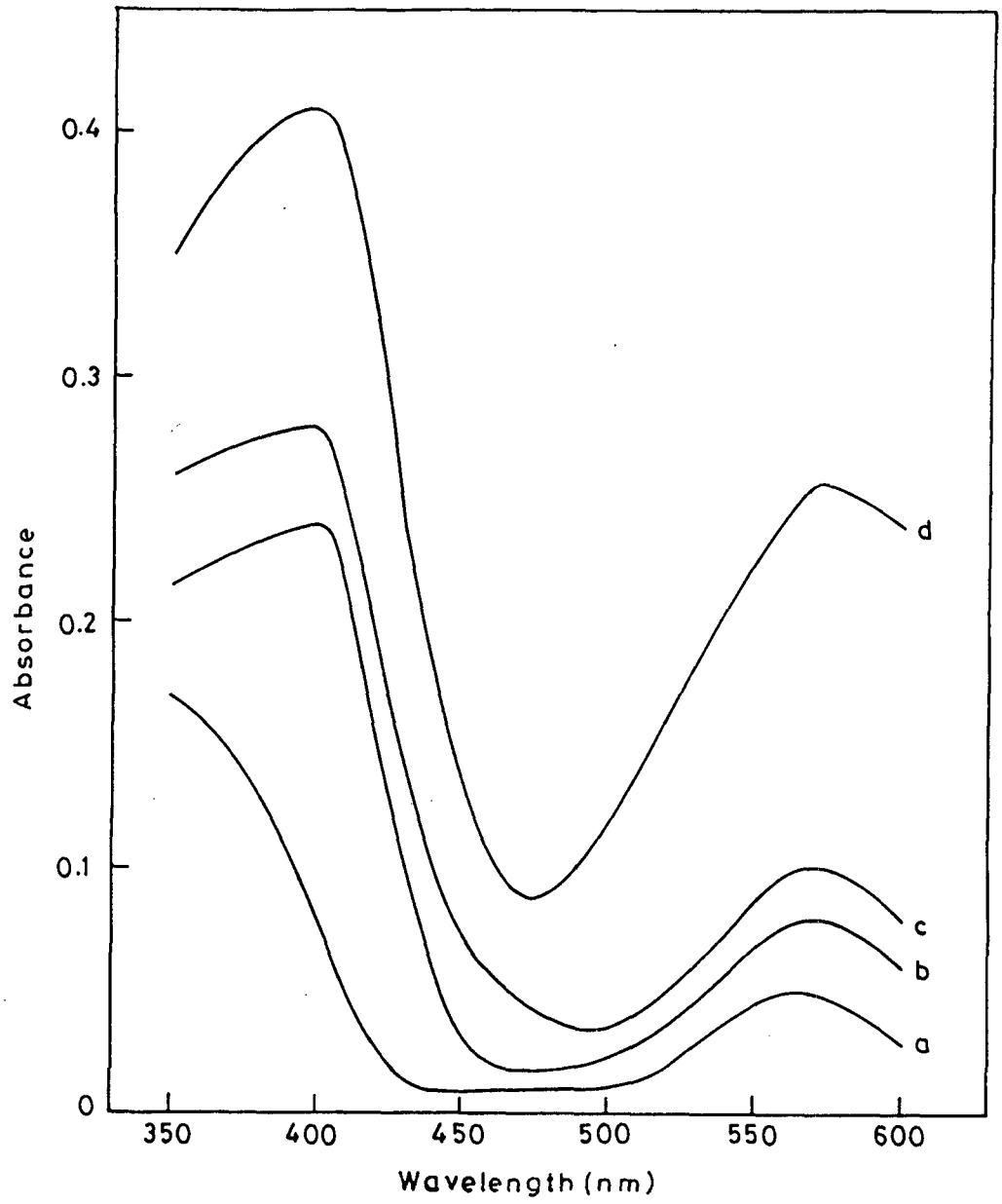


Fig. 2.10: Absorption spectra of the reaction product of glutamic acid with ninhydrin in absence of CTAB (a), 5.0 x 10^{-3} mol dm^{-3} CTAB (b), 10.0 x 10^{-3} mol dm^{-3} CTAB (c), 10.0 x 10^{-3} mol dm^{-3} CTAB + 10% DMSO (d). *Reaction conditions* : $[\text{glutamic acid}]_T = 1.0 \times 10^{-4}$ mol dm^{-3}, $[\text{ninhydrin}]_T = 5.0 \times 10^{-3}$ mol dm^{-3}, pH = 5.0, temp. = 80 °C.

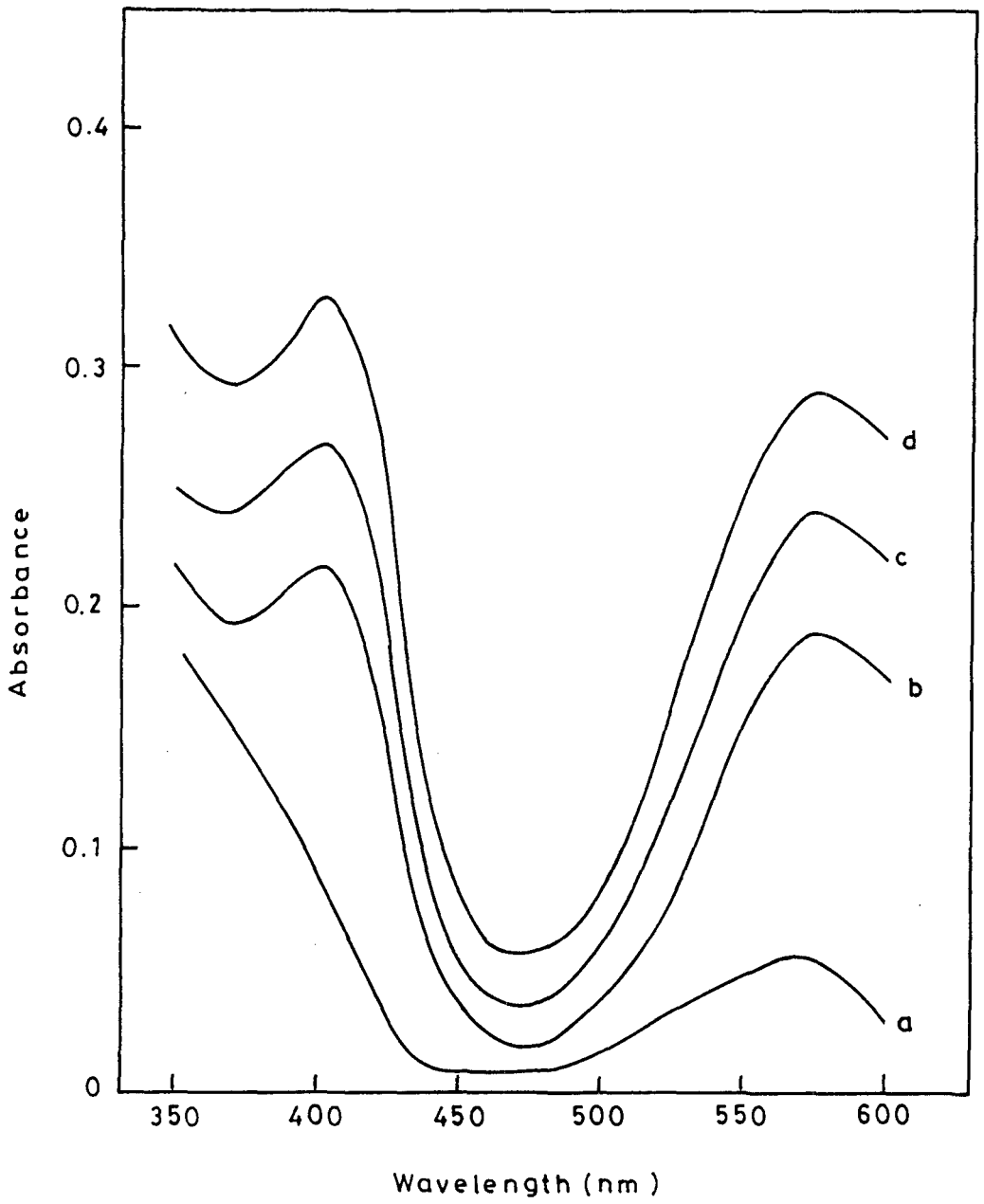


Fig. 2.11: Absorption spectra of the reaction product of arginine with ninhydrin in absence of CTAB (a), 5.0×10^{-3} mol dm^{-3} CTAB (b), 10.0×10^{-3} mol dm^{-3} CTAB (c), 10.0×10^{-3} mol dm^{-3} CTAB + 10% DMSO (d). *Reaction conditions* : $[arginine]_T = 1.0 \times 10^{-4}$ mol dm^{-3}, $[ninhydrin]_T = 5.0 \times 10^{-3}$ mol dm^{-3}, pH = 5.0, temp. = 80 °C.

Kinetic Measurements

The required solution of amino acid along with other reagents (when required) was taken in a three-necked vessel fitted with a double-surface water condenser to check any evaporation. The reaction flask was kept immersed in an oil bath set at the required temperature within ±0.1 °C. Required volume of thermally equilibrated ninhydrin solution was added to start the reaction. The zero-time was recorded after half of the addition of the ninhydrin solution. A slow stream of pure nitrogen gas (free from CO_2 and O_2) was passed through the reaction mixture for stirring and maintaining an inert atmosphere. The progress of the reaction was monitored spectrophotometrically by pipetting out aliquots at various time intervals and measuring the absorbance of the product at 570 nm,[169,170] using a Bausch and Lomb Spectronic-20 spectrophotometer. The [ninhydrin] was kept in excess in order to maintain *pseudo*-first-order conditions.

Values of *pseudo*-first-order rate constants (k_{obs} and k_{ψ}) were obtained from plots of log $(A_{\infty}-A_0)/(A_{\infty}-A_t)$ *vs.* time (t) by a least-squares regression analysis of the data. The values of absorbance at infinite time (A_{∞}) for each amino acid-ninhydrin system were obtained in the following manner. At the end of each kinetic run, 10 cm^3 of the solution mixture (after taking into a standard volumetric flask) was boiled for 2 min. It was then cooled to room temperature and, after adding buffer solution to compensate any volume loss, absorbance was recorded. However, in case

of methionine, as the absorbance started decreasing after some time (the time depended on the reaction conditions), the value of maximum absorbance was taken as A_{∞}. It should be mentioned here that the kinetic treatment of the data are, therefore, somewhat unreliable. As sulphur containing amino acids are known to cause blockage/decomposition of the purple-coloured product,[169] kinetic studies with methionine were not performed in micellar media.

The dependence of observed rate constants (k_{obs} in aqueous and k_{ψ} in micellar media) were obtained as a function of [amino acid], [ninhydrin], [surfactant], % solvent (v/v), temperature and pH. The results are recorded in Chapter 3. The rate constants obtained from multiple determinations agreed within ±4%.

Determination of cmc by Conductivity Measurements

The determination of cmc by conductivity measurements were carried out by using a conductivity bridge (ELICO, Hyderabad, India, TYPE CM 82T). First, the conductivity of the solvent was measured and then conductivity was recorded every time after addition of small volumes of the stock solution of CTAB and ensuring complete mixing. The specific conductivity was calculated by applying the solvent correction. The cmc values of surfactant (CTAB) in the presence and absence of reactants were obtained from the break points of nearly two straight line portions of the

specific conductivity versus surfactant concentration plots[171] (Figs. 2.12-2.17). The measurements were made at 25 and 80 °C under varying conditions, i.e., solvent being water, water + amino acid, water + ninhydrin, water + amino acid + ninhydrin. The results are recorded in Table 2.2.

TABLE 2.2

Critical micelle concentration (cmc) values of CTAB in absence and presence of various amino acids[a] and ninhydrin (5.0×10^{-3} mol dm^{-3}) at 25 and 80 °C.

Solution	10^4 cmc (mol dm^{-3})	
	25 °C	80 °C
water	9.8	16.4
ninhydrin	9.4	15.4
alanine	8.5	15.6
alanine + ninhydrin	8.2	15.1
threonine	9.3	15.8
threonine + ninhydrin	9.0	15.6
tyrosine	8.7	16.0
tyrosine + ninhydrin	8.3	15.6
glutamic acid	9.2	15.8
glutamic acid + ninhydrin	8.9	15.2
arginine	9.1	15.9
arginine + ninhydrin	9.0	15.5

[a]amino acid concentration was 1.0×10^{-4} mol dm^{-3} except for alanine (=3.0×10^{-4} mol dm^{-3}) and threonine (=2.0×10^{-4} mol dm^{-3}).

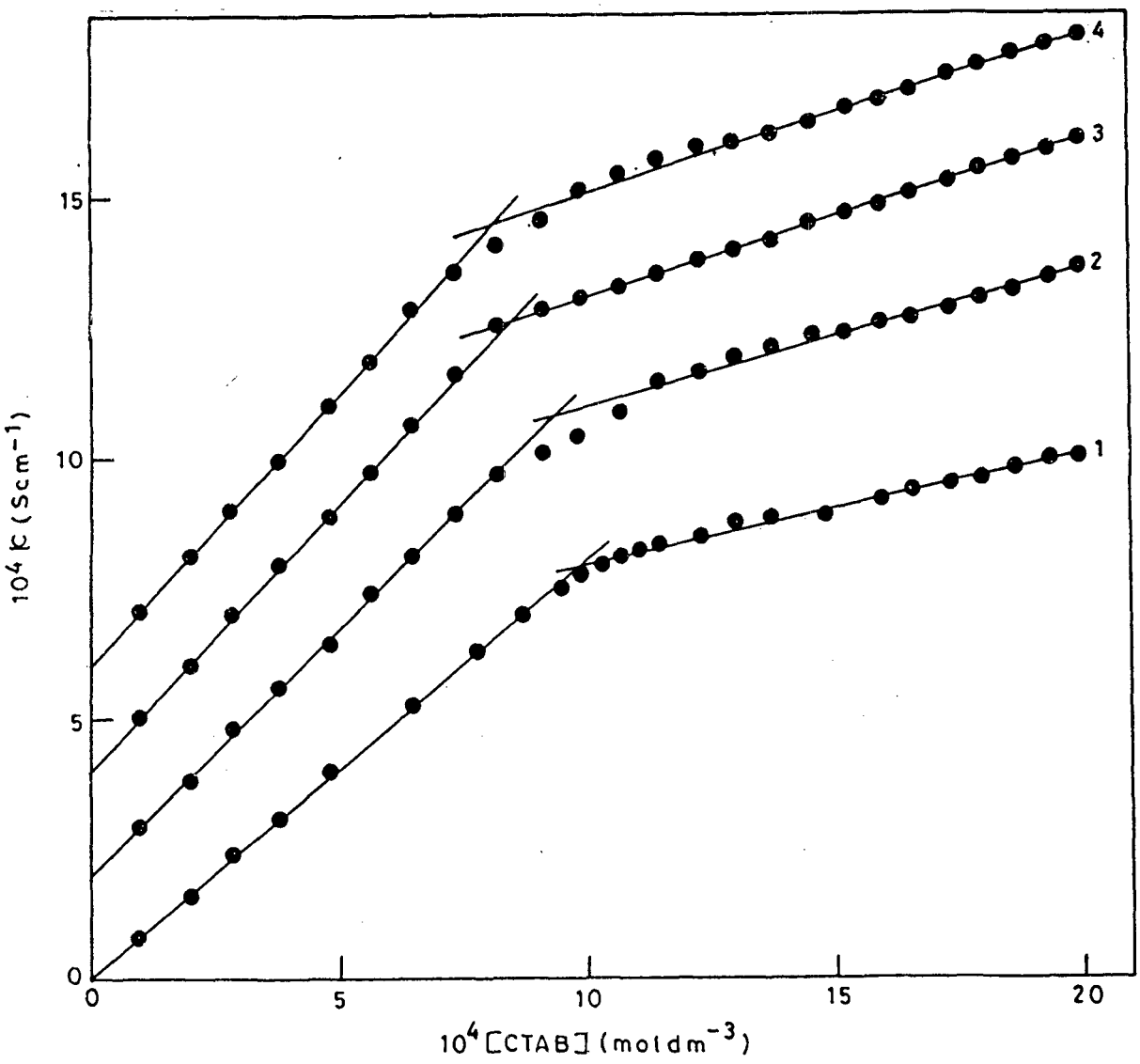


Fig. 2.12: Variation of specific conductivity (κ) with CTAB concentration at 25 °C : in water (1), in water in the presence of 5.0 x 10^{-3} mol dm^{-3} ninhydrin (2), in water in the presence of 3.0 x 10^{-4} mol dm^{-3} alanine (3), in water in the presence of 3.0 x 10^{-4} mol dm^{-3} alanine and 5.0 x 10^{-3} mol dm^{-3} ninhydrin (4). The scale shown is for curve 1. Curves 2, 3, 4 have been shifted upwards by 2, 4, 6 scale units (1 x 10^{-4} S cm^{-1}), respectively.

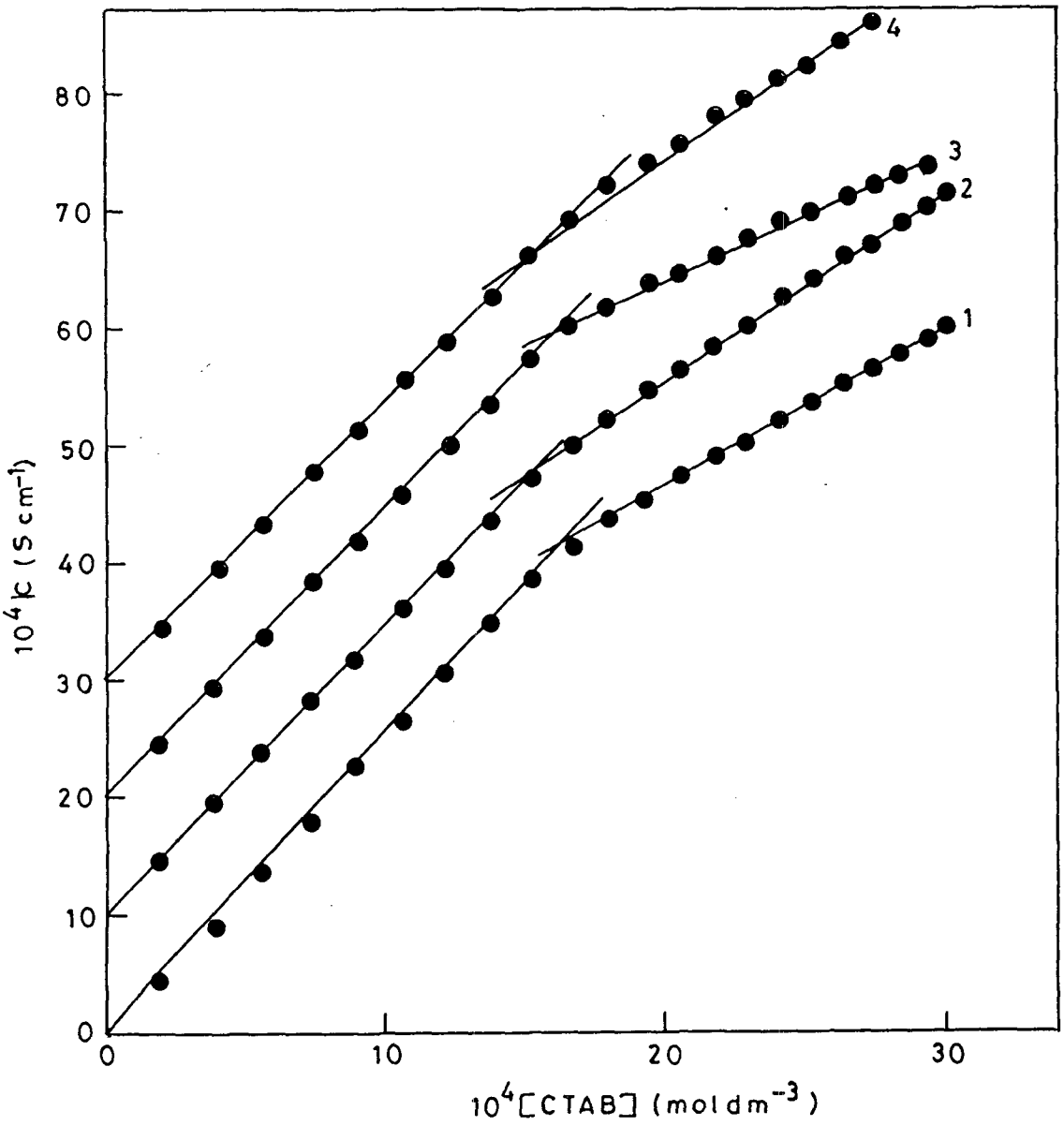


Fig. 2.13: Variation of specific conductivity (κ) with CTAB concentration at 80 °C in water (1), in water in the presence of 5.0 x 10^{-3} mol dm^{-3} ninhydrin (2), in water in the presence of 3.0 x 10^{-4} mol dm^{-3} alanine (3), in water in the presence of 3.0 x 10^{-4} mol dm^{-3} alanine and 5.0 x 10^{-3} mol dm^{-3} ninhydrin (4). The scale shown is for curve 1. Curves 2, 3, 4 have been shifted upwards by 10, 20, 30 scale units (1 x 10^{-4} S cm^{-1}), respectively.

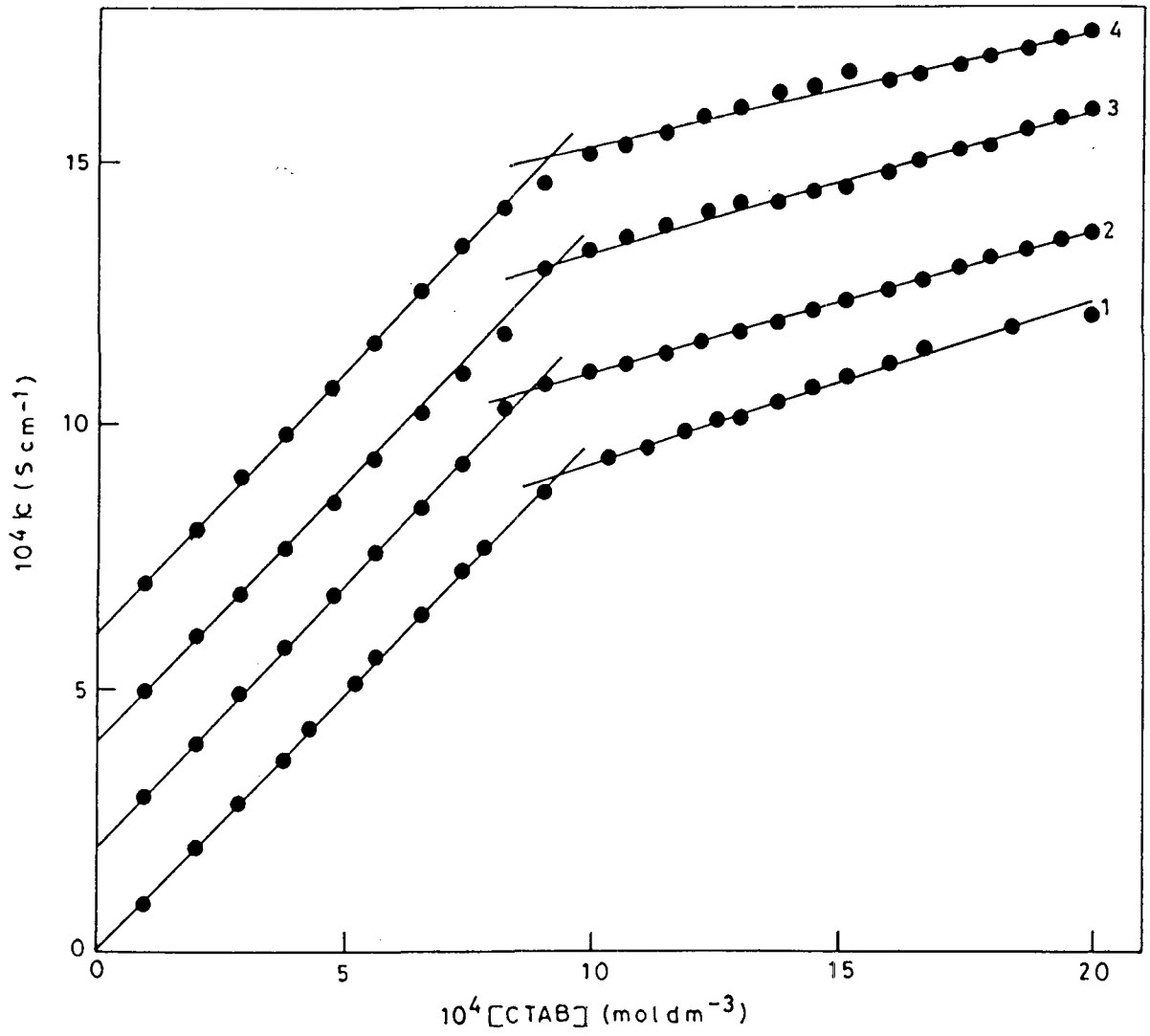


Fig. 2.14: Variation of specific conductivity (κ) with CTAB concentration at 25 °C : in water in the presence of 2.0 x 10^{-4} mol dm^{-3} threonine (1), in water in the presence of 1.0 x 10^{-4} mol dm^{-3} tyrosine (2), in water in the presence of 1.0 x 10^{-4} mol dm^{-3} glutamic acid (3), in water in the presence of 1.0 x 10^{-4} mol dm^{-3} arginine (4). The scale shown is for curve 1. Curves 2, 3, 4 have been shifted upwards by 2, 4, 6 scale units (1 x 10^{-4} S cm^{-1}), respectively.

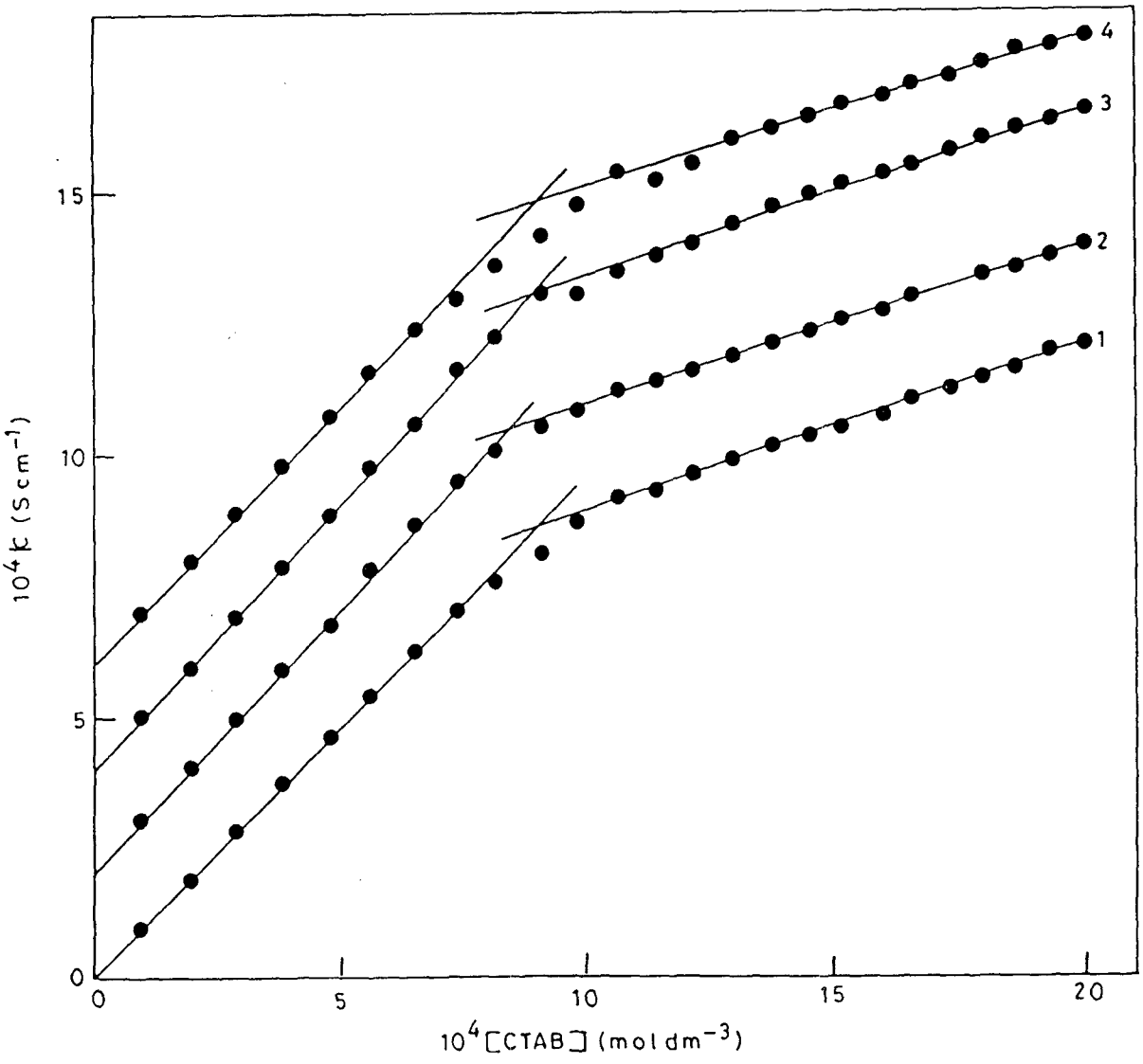


Fig. 2.15: Variation of specific conductivity (κ) with CTAB concentration at 25 °C : in water in the presence of 2.0 x 10^{-4} mol dm^{-3} threonine and 5.0 x 10^{-3} mol dm^{-3} ninhydrin (1), in water in the presence of 1.0 x 10^{-4} mol dm^{-3} tyrosine and 5.0 x 10^{-3} mol dm^{-3} ninhydrin (2), in water in the presence of 1.0 x 10^{-4} mol dm^{-3} glutamic acid and 5.0 x 10^{-3} mol dm^{-3} ninhydrin (3), in water in the presence of 1.0 x 10^{-4} mol dm^{-3} arginine and 5.0 x 10^{-3} mol dm^{-3} ninhydrin (4). The scale shown is for curve 1. Curves 2, 4, 6 have been shifted upwards by 2, 4, 6 scale units (1 x 10^{-4} S cm^{-1}), respectively.

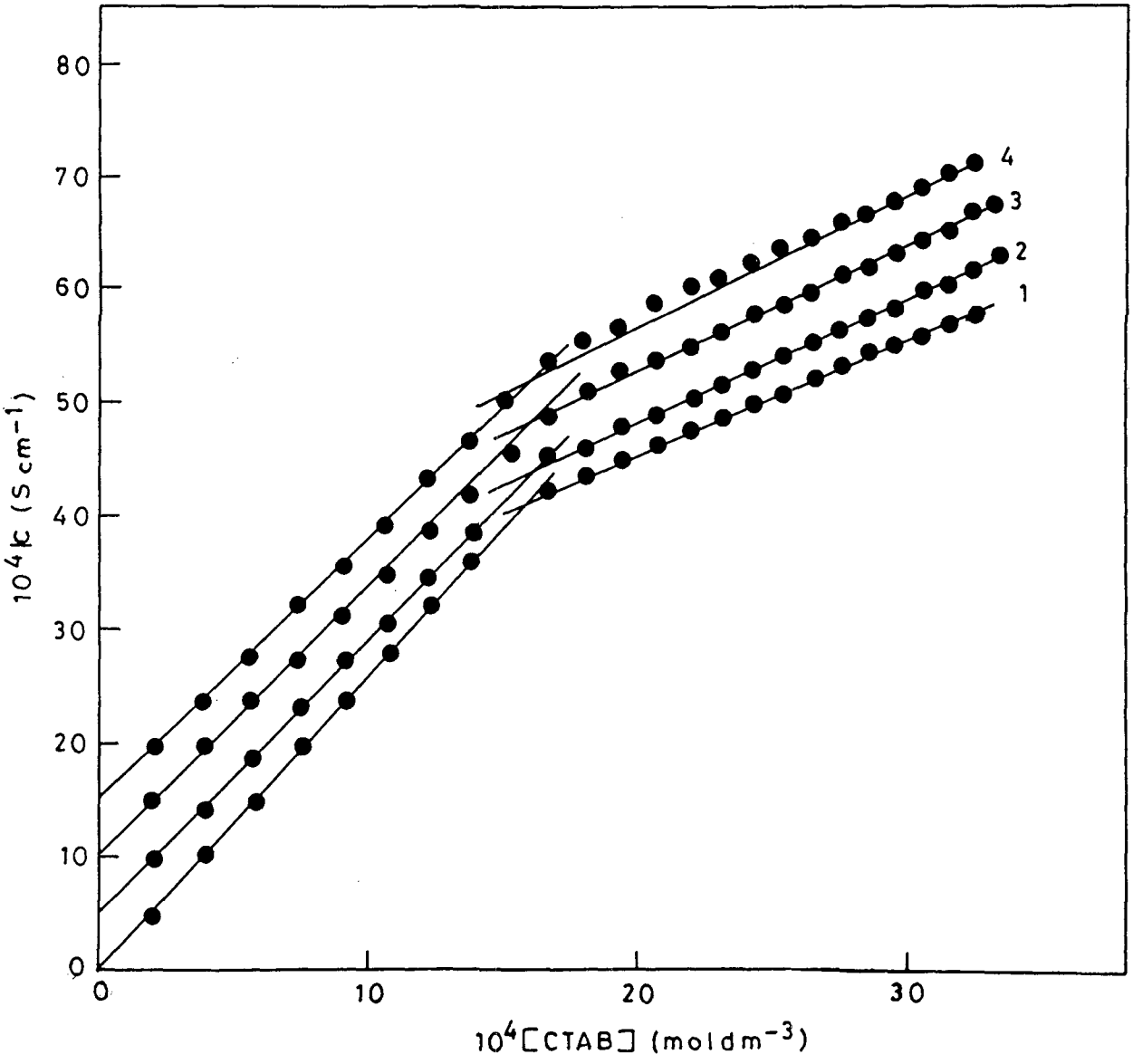


Fig. 2.16: Variation of specific conductivity (κ) with CTAB concentration at 80 °C : in water in the presence of 2.0 x 10^{-4} mol dm^{-3} threonine (1), in water in the presence of 1.0 x 10^{-4} mol dm^{-3} tyrosine (2), in water in the presence of 1.0 x 10^{-4} mol dm^{-3} glutamic acid (3), in water in the presence of 1.0 x 10^{-4} mol dm^{-3} arginine (4). The scale shown is for curve 1. Curves 2, 3, 4 have been shifted upwards by 5, 10, 15 scale units (1 x 10^{-4} S cm^{-1}), respectively.

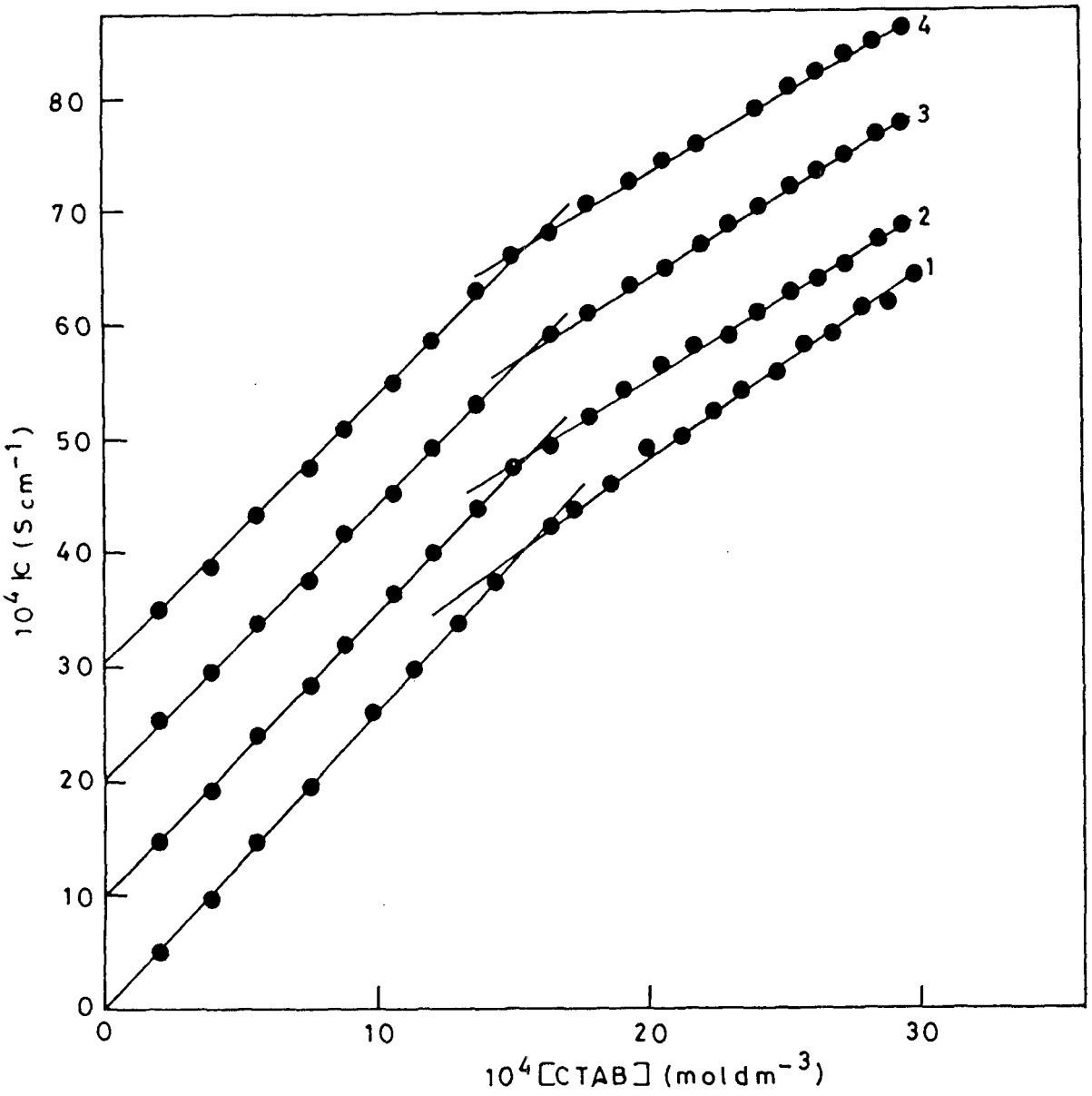


Fig. 2.17: Variation of specific conductivity (κ) with CTAB concentration at 80 °C : in water in the presence of 2.0 x 10^{-4} mol dm^{-3} threonine and 5.0 x 10^{-3} mol dm^{-3} ninhydrin (1), in water in the presence of 1.0 x 10^{-4} mol dm^{-3} tyrosine and 5.0 x 10^{-3} mol dm^{-3} ninhydrin (2), in water in the presence of 1.0 x 10^{-4} mol dm^{-3} glutamic acid and 5.0 x 10^{-3} mol dm^{-3} ninhydrin (3), in water in the presence of 1.0 x 10^{-4} mol dm^{-3} arginine and 5.0 x 10^{-3} mol dm^{-3} ninhydrin (4). The scale shown is for curve 1. Curves 2, 3, 4 have been shifted upwards by 10, 20, 30 scale units (1 x 10^{-4} S cm^{-1}), respectively.

CHAPTER - 3

RESULTS AND DISCUSSION

A. Results

The visible spectra of the purple-coloured product (formed by the reaction of amino acids with ninhydrin) were recorded after completion of the reactions in the absence and presence of CTAB in aqueous and aqueous-organic solvents. The spectra consist of sharp peaks at λ_{max} = 400, 570 nm in all media, which is in line with the literature value.[169,170] The absorption maximum at 570 nm is generally used for quantitative and qualitative studies with the ninhydrin reaction. The observation that the absorption maxima did not change in the presence of CTAB and/or organic solvents leads to the conclusion that the presence of CTAB and/or organic solvents did not bring any change in the product formation. The same product is, therefore, formed in the presence of CTAB and solvents as in aqueous solution. It has also been observed that the reactions of ninhydrin and amino acids are catalyzed in presence of CTAB.

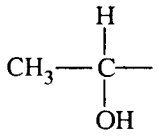
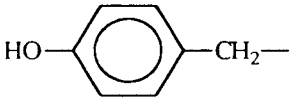
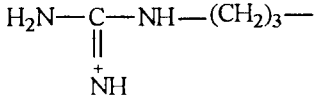
We have performed systematic kinetic and mechanistic studies of the formation of Ruhemann's purple in the absence and presence of cationic micelles both in aqueous and aqueous-organic media. The acid dissociation constants (pK_a's) of the amino acids used, their symbols, and abbreviations are summarized in Table 3.1.

Effect of pH

The solution pH plays a pivotal role in the amino acid-ninhydrin reaction; this being the reason that the effect of pH on the rate of amino

TABLE 3.1

Amino acids ($RCH(NH_2)COOH$) used in the present investigation and their pK_a-values at 25 °C[a]

Amino acid	Three-letter abbreviation	R	$pK_{a(COOH)}$	$pK_{a(\alpha\text{-}\overset{+}{N}H3)}$	$pK_{a(R)}$
DL-Alanine	Ala	CH_3-	2.35	9.87	
DL-Methionine	Met	$CH_3-S-CH_2-CH_2-$	2.28	9.21	
DL-Threonine	Thr		2.63	10.43	
L-Tyrosine	Tyr		2.20	9.11	10.07
L-Glutamic acid	Glu	$HOOC-CH_2-CH_2-$	2.10	4.07	9.47
L-Arginine	Arg		2.01	9.04	12.48

a) R.C. Weast, "CRC Handbook of Chemistry and Physics", CRC Press, Inc., Florida, 58th ed., 1977-1978.

acid-ninhydrin reactions was studied in the pH range 3.6 to 6.0. The results are recorded in Tables 3.2-3.4 and shown graphically in Figs. 3.1-3.6. The formation of Ruhemann's purple was known to be dependent on pH and humidity, and complete development was known to require heating.[172] The optimal pH had been determined to be approximately 5.0. That is why the whole study of this thesis was undertaken at pH 5.0.

Dependence of the Reaction Rate on Amino Acid Concentration

The k_{obs} and k_{ψ} were determined at different initial amino acid concentrations with a view to find the order with respect to [amino acid]. Various concentrations of amino acids were used at fixed values of $[ninhydrin]_T$ along with fixed $[CTAB]_T$ (when required) and pH (5.0) at 80 °C. It was observed that the values of rate constants (both k_{obs} and k_{ψ}) were independent of the initial amino acid concentration in both the media (Tables 3.5-3.15). This shows that the order of the reaction with respect to [amino acid] is unity. The rate law would then be

$$\text{rate} = d[\text{product}]/dt = k[\text{amino acid}]_T \; (k = k_{obs} \text{ or } k_{\psi}) \qquad (3.1)$$

Dependence of Reaction Rate on Ninhydrin Concentration

Both in the absence and presence of CTAB, the dependence of rate constants (k_{obs} and k_{ψ}) was obtained at different $[ninhydrin]_T$ from 5.0-40.0 x 10^{-3} mol dm^{-3} (except DL-methionine where [ninhydrin] ranged from 5.0 - 30.0 x 10^{-3} mol dm^{-3}) at constant $[amino\ acid]_T$, $[CTAB]_T$

TABLE 3.2

Effect of pH on the *pseudo*-first-order rate constants (k_{obs} or k_{ψ}) for the reaction of amino acid with ninhydrin.

Reaction Conditions :

$[\text{amino acid}]_T$ = 3.0×10^{-4} mol dm^{-3}

$[\text{ninhydrin}]_T$ = 5.0×10^{-3} mol dm^{-3}

Temperature = 80 °C

pH	DL-alanine		DL-methionine
	$10^5 k_{obs}$ (s^{-1})	$10^5 k_{\psi}$ [a] (s^{-1})	$10^5 k_{obs}$ (s^{-1})
3.6	no reaction	3.7	no reaction
4.0	0.1	4.5	2.2
4.5	1.0	6.7	2.7
5.0	5.4	13.4	10.6
5.5	7.5	17.9	11.1
6.0	8.0	19.2	10.9

[a] $[\text{CTAB}]_T = 1.0 \times 10^{-2}$ mol dm^{-3}.

TABLE 3.3

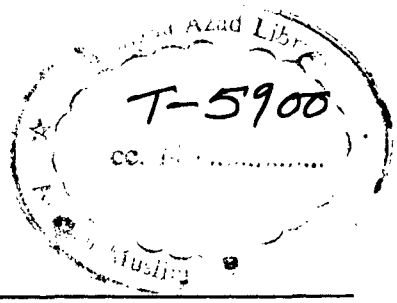
Effect of pH on the *pseudo*-first-order rate constants (k_{obs} or k_{ψ}) for the reaction of amino acid with ninhydrin.

Reaction Conditions :

$[ninhydrin]_T$ = 5.0×10^{-3} mol dm^{-3}

pH = 5.0

Temperature = 80 °C



pH	DL-threonine[a]		L-tyrosine[b]	
	$10^5 k_{obs}$ (s^{-1})	$10^5 k_{\psi}$[c] (s^{-1})	$10^5 k_{obs}$ (s^{-1})	$10^5 k_{\psi}$[c] (s^{-1})
3.6	2.2	4.2	no reaction	2.1
4.0	3.1	6.0	0.9	3.5
4.5	4.6	11.2	2.2	7.5
5.0	7.8	17.4	11.5	14.3
5.5	6.6	18.9	12.5	16.8
6.0	9.6	20.1	13.3	18.5

[a] $[DL\text{-threonine}]_T = 2.0 \times 10^{-4}$ mol dm^{-3}.

[b] $[L\text{-tyrosine}]_T = 1.0 \times 10^{-4}$ mol dm^{-3}.

[c] $[CTAB]_T = 2.0 \times 10^{-2}$ mol dm^{-3}.

TABLE 3.4

Effect of pH on the *pseudo*-first-order rate constants (k_{obs} or k_{ψ}) for the reaction of amino acid with ninhydrin.

Reaction Conditions :

$[\text{amino acid}]_T$ = 1.0×10^{-4} mol dm^{-3}

$[\text{ninhydrin}]_T$ = 5.0×10^{-3} mol dm^{-3}

Temperature = 80 °C

	L-glutamic acid		L-arginine	
pH	$10^5 k_{obs}$ (s^{-1})	$10^5 k_{\psi}$ [a] (s^{-1})	$10^5 k_{obs}$ (s^{-1})	$10^5 k_{\psi}$ [b] (s^{-1})
3.6	0.6	2.7	0.7	1.7
4.0	1.2	3.3	1.3	3.0
4.5	1.4	4.8	1.8	4.7
5.0	3.5	8.3	4.3	10.0
5.5	4.3	10.7	10.8	11.8
6.0	5.8	14.6	11.8	15.7

[a] $[\text{CTAB}] = 2.0 \times 10^{-2}$ mol dm^{-3}.

[b] $[\text{CTAB}] = 1.0 \times 10^{-2}$ mol dm^{-3}.

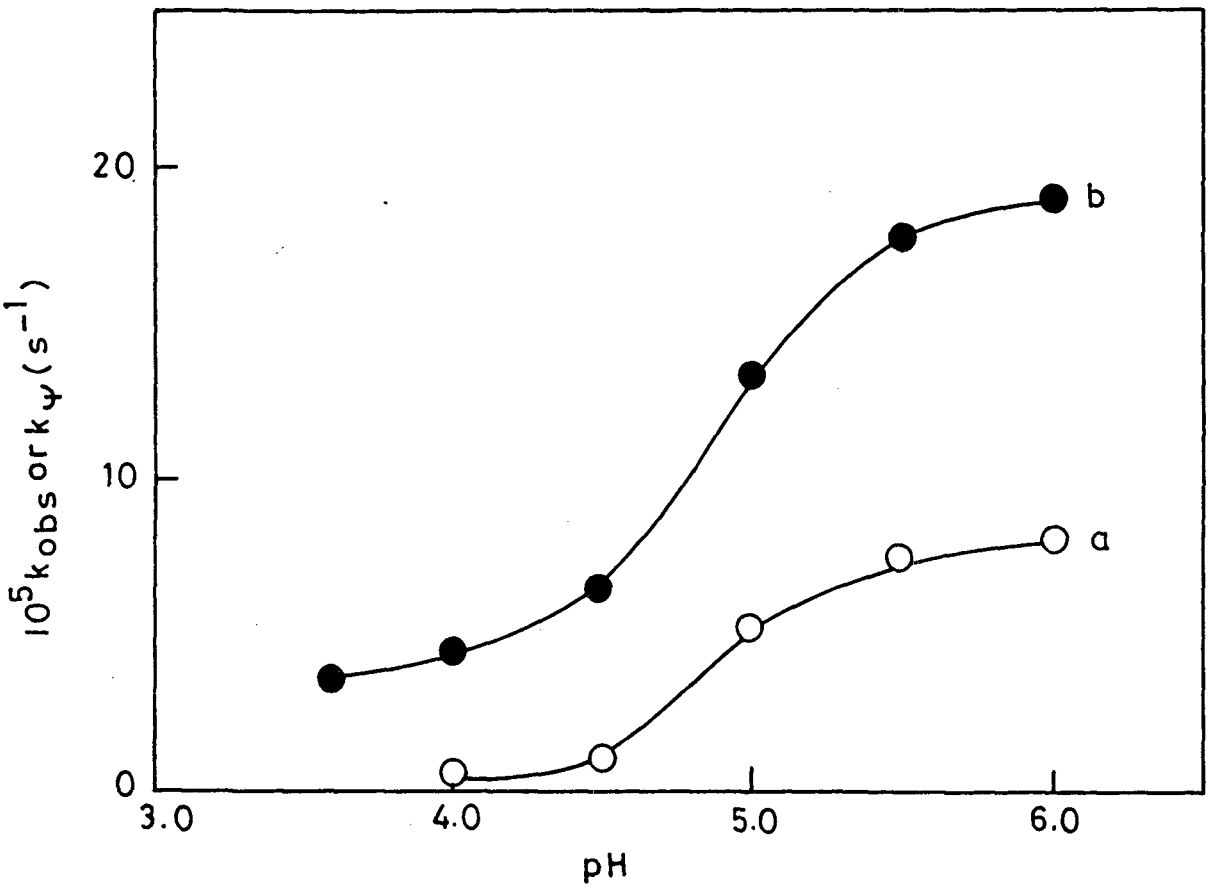


Fig. 3.1: Effect of pH on the reaction rate of alanine with ninhydrin in absence of CTAB (a), and in presence of 1.0 x 10^{-2} mol dm^{-3} CTAB (b). *Reaction conditions*: $[alanine]_T = 3.0 \times 10^{-4}$ mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, temp. = 80 °C.

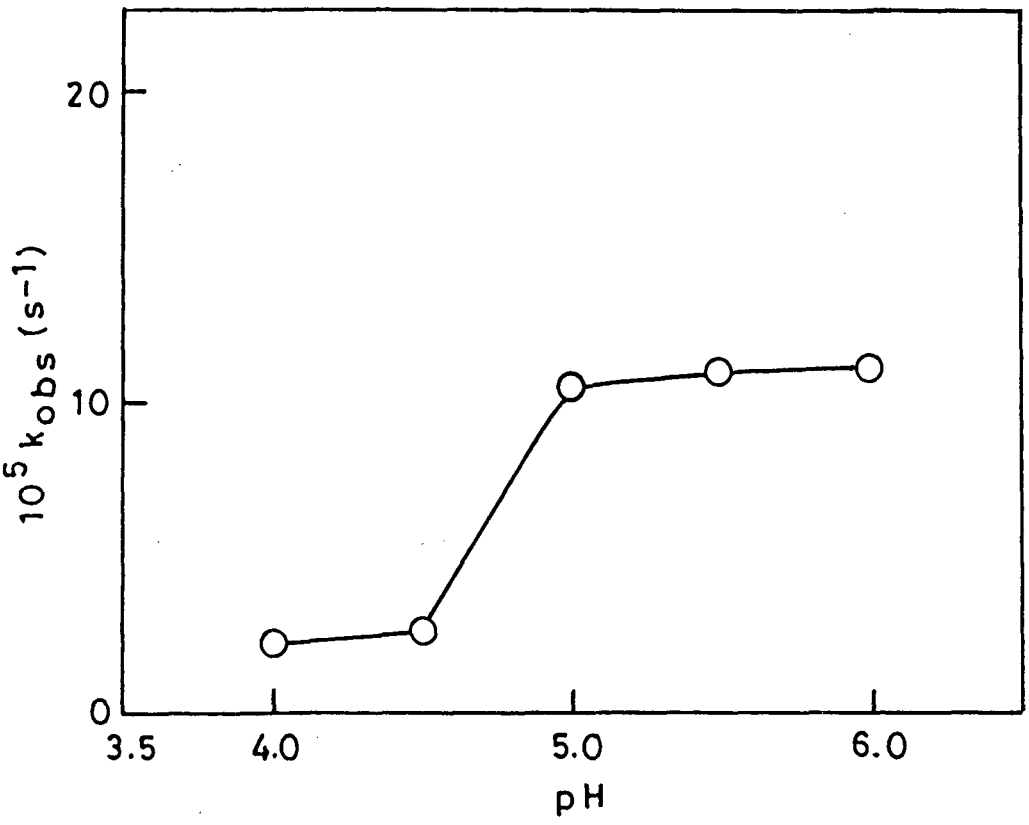


Fig. 3.2: Effect of pH on the reaction rate of methionine with ninhydrin. *Reaction conditions* : $[methionine]_T$ = 3.0 x 10^{-4} mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, temp. = 80 °C.

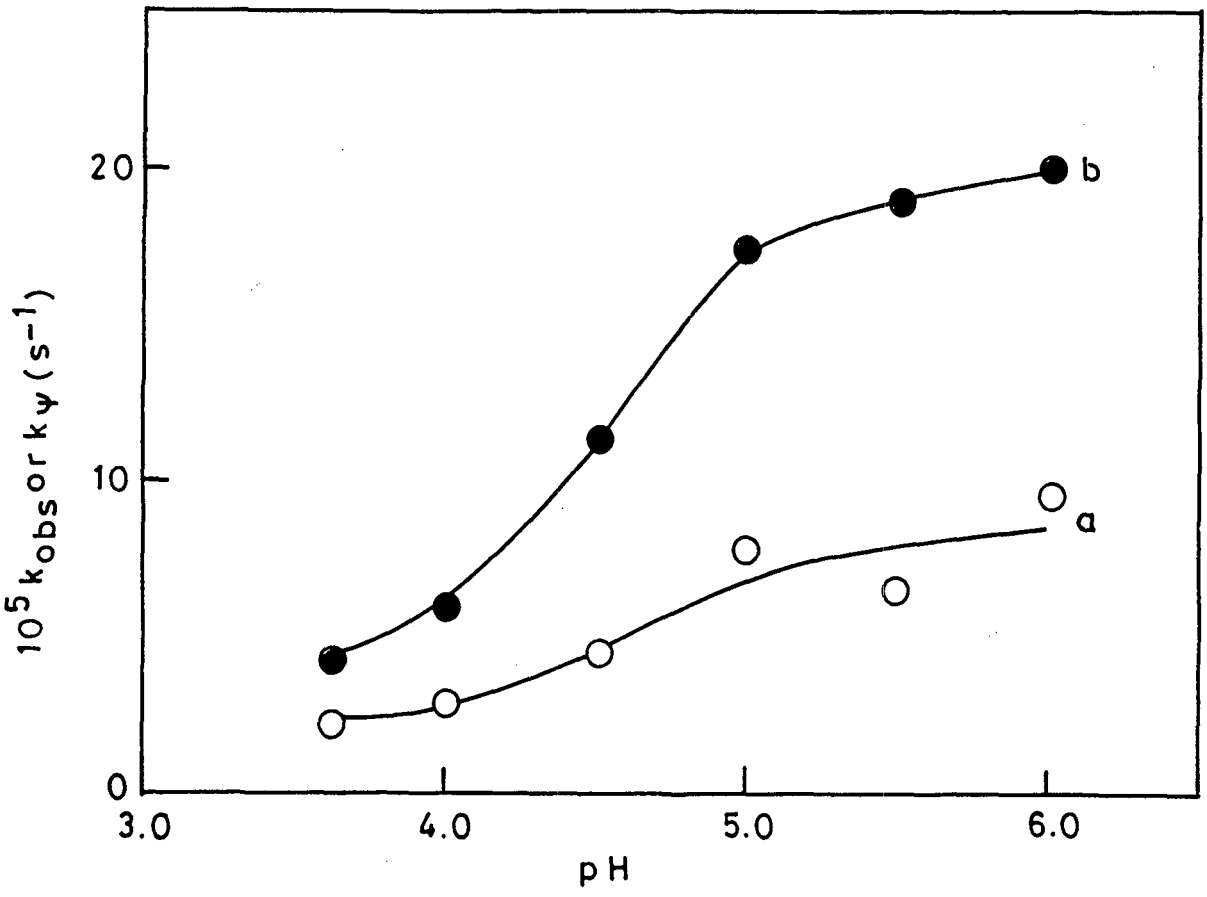


Fig. 3.3: Effect of pH on the reaction rate of threonine with ninhydrin in absence of CTAB (a), and in presence of 2.0 x 10^{-2} mol dm^{-3} CTAB (b). *Reaction conditions* : $[threonine]_T$ = 2.0 x 10^{-4} mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, temp. = 80 °C.

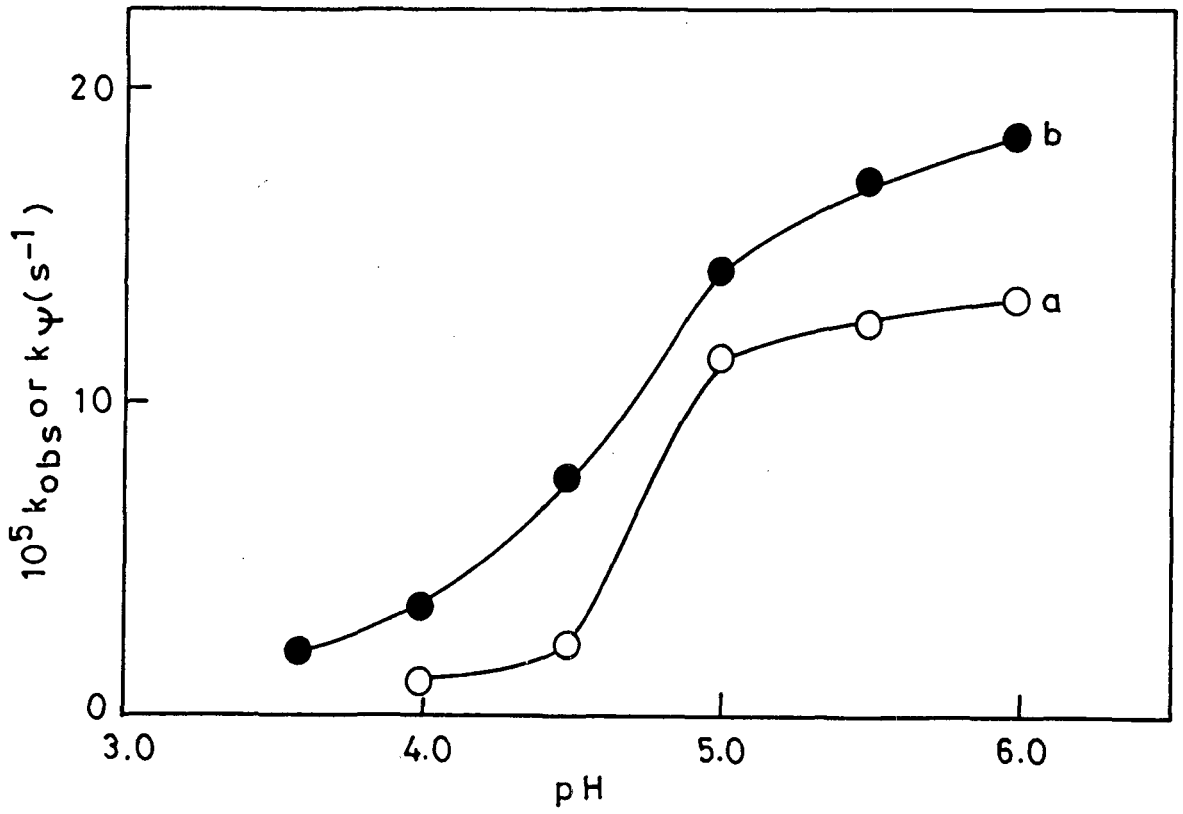


Fig. 3.4: Effect of pH on the reaction rate of tyrosine with ninhydrin in absence of CTAB (a), and in presence of 2.0 x 10^{-2} mol dm^{-3} CTAB (b). *Reaction conditions* : $[tyrosine]_T$ = 1.0 x 10^{-4} mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, temp. = 80 °C.

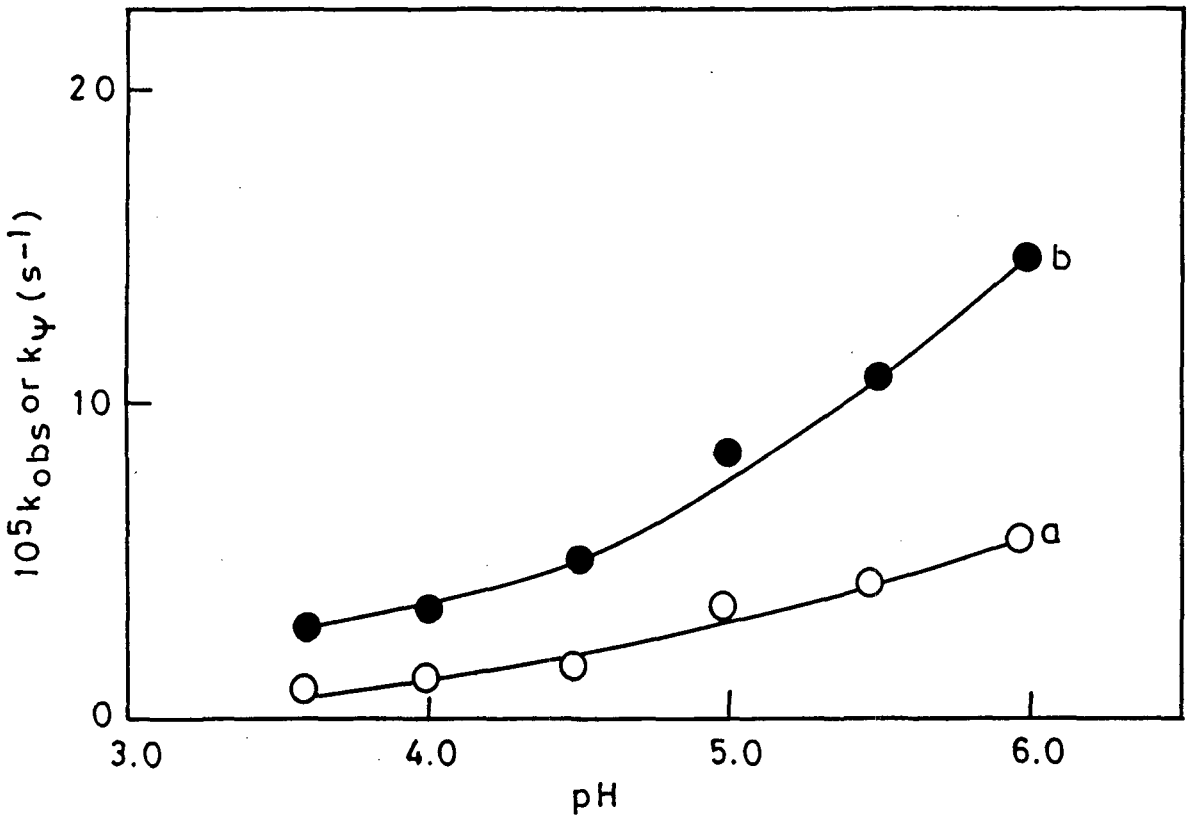


Fig. 3.5: Effect of pH on the reaction rate of glutamic acid with ninhydrin in absence of CTAB (a), and in presence of 2.0×10^{-2} mol dm^{-3} CTAB (b). *Reaction conditions* : $[\text{glutamic acid}]_T$ = 1.0×10^{-4} mol dm^{-3}, $[\text{ninhydrin}]_T$ = 5.0×10^{-3} mol dm^{-3}, temp. = 80 °C.

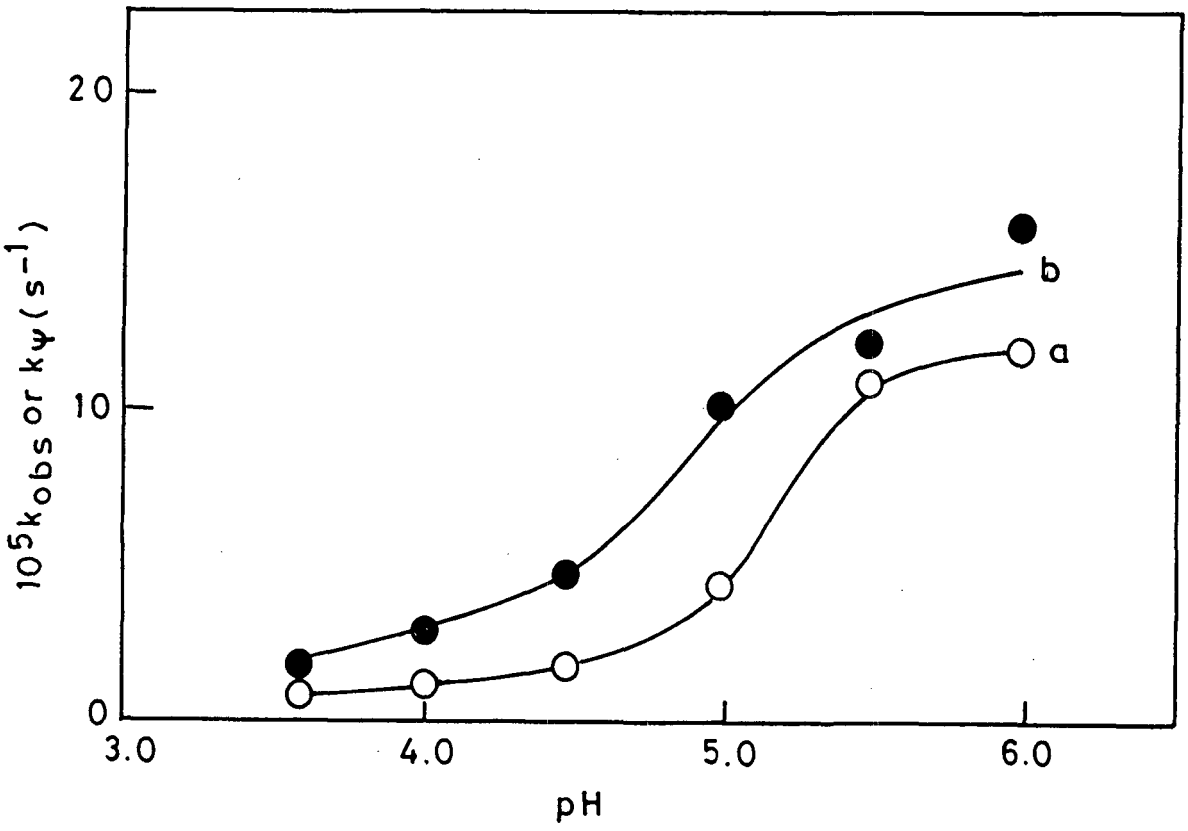


Fig. 3.6: Effect of pH on the reaction rate of arginine with ninhydrin in absence of CTAB (a), and in presence of 1.0×10^{-2} mol dm^{-3} CTAB (b). *Reaction conditions* : $[arginine]_T = 1.0 \times 10^{-4}$ mol dm^{-3}, $[ninhydrin]_T = 5.0 \times 10^{-3}$ mol dm^{-3}, temp. = 80 °C.

TABLE 3.5

Effect of [alanine] and [ninhydrin] on the *pseudo*-first-order rate constants (k_{obs}) for the reaction of alanine with ninhydrin.

Reaction conditions :

pH = 5.0

Temperature = 80 °C

$10^4[Ala]_T$ (mol dm^{-3})	$10^2[ninhydrin]_T$ (mol dm^{-3})	10^5k_{obs} (s^{-1})	10^5k_{cal} (s^{-1})
2.5	0.5	5.1	
3.0	0.5	5.4	
3.5	0.5	5.2	
4.0	0.5	5.1	
4.5	0.5	5.9	
3.0	0.5	5.4	5.6
3.0	1.0	11.5	10.5
3.0	1.5	17.4	15.0
3.0	2.0	20.0	19.0
3.0	2.5	21.8	22.6
3.0	3.0	24.0	26.0
3.0	3.5	26.0	29.0
3.0	4.0	27.1	31.8

TABLE 3.6

Effect of [alanine] and [ninhydrin] on the *pseudo*-first-order rate constants (k_ψ) for the reaction of alanine with ninhydrin.

Reaction conditions :

$[CTAB]_T$ = 1.0×10^{-2} mol dm^{-3}

pH = 5.0

Temperature = 80 °C

$10^4[Ala]_T$ (mol dm^{-3})	$10^2[ninhydrin]_T$ (mol dm^{-3})	$10^5 k_\psi$ (s^{-1})
2.5	0.5	13.2
3.0	0.5	13.4
3.5	0.5	13.3
4.0	0.5	13.7
4.5	0.5	13.4
3.0	0.5	13.4
3.0	1.0	22.7
3.0	1.5	32.6
3.0	2.0	44.2
3.0	2.5	50.6
3.0	3.0	56.1
3.0	3.5	58.5
3.0	4.0	60.8

TABLE 3.7

Effect of [methionine] and [ninhydrin] on the *pseudo*-first-order rate constants (k_{obs}) for the reaction of methionine with ninhydrin.

Reaction conditions :

pH = 5.0

Temperature = 80 °C

$10^4[Met]_T$ (mol dm^{-3})	$10^2[ninhydrin]_T$ (mol dm^{-3})	$10^5 k_{obs}$ (s^{-1})
2.6	0.5	10.0
3.0	0.5	10.6
3.4	0.5	10.6
3.8	0.5	11.4
4.2	0.5	11.7
4.6	0.5	11.7
3.0	0.5	10.6
3.0	1.0	17.7
3.0	1.25	26.5
3.0	1.5	30.6
3.0	2.0	44.0
3.0	2.5	52.3
3.0	3.0	77.4

TABLE 3.8

Effect of [threonine] and [ninhydrin] on the *pseudo*-first-order rate constants (k_{obs}) for the reaction of threonine with ninhydrin.

Reaction conditions :

pH = 5.0

Temperature = 80 °C

$10^4[Thr]_T$ (mol dm^{-3})	$10^2[ninhydrin]_T$ (mol dm^{-3})	10^5k_{obs} (s^{-1})	10^5k_{cal} (s^{-1})
2.0	0.5	7.8	
2.5	0.5	7.2	
3.0	0.5	7.9	
3.5	0.5	7.0	
4.0	0.5	7.2	
2.0	0.5	7.8	7.8
2.0	1.0	15.1	14.6
2.0	1.5	20.5	20.5
2.0	2.0	24.7	25.7
2.0	2.5	34.4	30.4
2.0	3.0	35.5	34.5
2.0	3.5	36.8	38.2
2.0	4.0	37.9	41.5

TABLE 3.9

Effect of [threonine] and [ninhydrin] on the *pseudo*-first-order rate constants (k_ψ) for the reaction of threonine with ninhydrin.

Reaction conditions :

$[CTAB]_T$ = 2.0×10^{-2} mol dm^{-3}

pH = 5.0

Temperature = 80 °C

$10^4[Thr]_T$ (mol dm^{-3})	$10^2[ninhydrin]_T$ (mol dm^{-3})	$10^5 k_\psi$ (s^{-1})
2.0	0.5	17.4
2.5	0.5	17.3
3.0	0.5	17.0
3.5	0.5	17.4
4.0	0.5	17.6
2.0	0.5	17.4
2.0	1.0	32.8
2.0	1.5	47.3
2.0	2.0	57.4
2.0	2.5	73.7
2.0	3.0	85.6
2.0	3.5	89.0
2.0	4.0	92.5

TABLE 3.10

Effect of [tyrosine] and [ninhydrin] on the *pseudo*-first-order rate constants (k_{obs}) for the reaction of tyrosine with ninhydrin.

Reaction conditions :

pH = 5.0

Temperature = 80 °C

$10^4[Tyr]_T$ (mol dm^{-3})	$10^2[ninhydrin]_T$ (mol dm^{-3})	10^5k_{obs} (s^{-1})	10^5k_{cal} (s^{-1})
1.0	0.5	11.5	
1.5	0.5	11.6	
2.0	0.5	11.3	
2.5	0.5	11.3	
3.0	0.5	11.0	
3.5	0.5	11.6	
1.0	0.5	11.5	11.5
1.0	1.0	16.3	18.1
1.0	1.5	20.7	22.8
1.0	2.0	28.2	26.3
1.0	2.5	31.0	29.0
1.0	3.0	32.7	31.0
1.0	3.5	33.2	32.7
1.0	4.0	34.6	34.1

TABLE 3.11

Effect of [tyrosine] and [ninhydrin] on the *pseudo*-first-order rate constants (k_ψ) for the reaction of tyrosine with ninhydrin.

Reaction conditions :

$[CTAB]_T$ = 2.0×10^{-2} mol dm^{-3}

pH = 5.0

Temperature = 80 °C

$10^4[Tyr]_T$ (mol dm^{-3})	$10^2[ninhydrin]_T$ (mol dm^{-3})	$10^5 k_\psi$ (s^{-1})
1.0	0.5	14.3
1.5	0.5	14.5
2.0	0.5	14.2
2.5	0.5	13.9
3.0	0.5	14.2
3.5	0.5	14.8
1.0	0.5	14.3
1.0	1.0	25.1
1.0	1.5	34.2
1.0	2.0	40.3
1.0	2.5	45.6
1.0	3.0	49.2
1.0	3.5	51.5
1.0	4.0	52.7

TABLE 3.12

Effect of [glutamic acid] and [ninhydrin] on the *pseudo*-first-order rate constants (k_{obs}) for the reaction of glutamic acid with ninhydrin.

Reaction conditions :

pH = 5.0

Temperature = 80 °C

$10^4[Glu]_T$ (mol dm^{-3})	$10^2[ninhydrin]_T$ (mol dm^{-3})	10^5k_{obs} (s^{-1})	10^5k_{cal} (s^{-1})
1.0	0.5	3.5	
1.5	0.5	3.6	
2.0	0.5	3.7	
2.5	0.5	3.2	
3.0	0.5	3.5	
3.5	0.5	3.4	
1.0	0.5	3.5	3.6
1.0	0.5	6.8	6.7
1.0	1.5	10.9	9.5
1.0	2.0	13.8	12.0
1.0	2.5	14.4	14.2
1.0	3.0	15.2	16.2
1.0	3.5	15.7	18.0
1.0	4.0	16.2	19.7

TABLE 3.13

Effect of [glutamic acid] and [ninhydrin] on the *pseudo*-first-order rate constants (k_ψ) for the reaction of glutamic acid with ninhydrin.

Reaction conditions :

$[CTAB]_T$ = 2.0×10^{-2} mol dm^{-3}

pH = 5.0

Temperature = 80 °C

$10^4[Glu]_T$ (mol dm^{-3})	$10^2[ninhydrin]_T$ (mol dm^{-3})	10^5k_ψ (s^{-1})
1.0	0.5	8.3
1.5	0.5	8.0
2.0	0.5	8.5
2.5	0.5	8.5
3.0	0.5	8.7
3.5	0.5	8.1
1.0	0.5	8.3
1.0	1.0	12.0
1.0	1.5	17.4
1.0	2.0	24.0
1.0	2.5	30.1
1.0	3.0	32.4
1.0	3.5	33.9
1.0	4.0	35.6

TABLE 3.14

Effect of [arginine] and [ninhydrin] on the *pseudo*-first-order rate constants (k_{obs}) for the reaction of arginine with ninhydrin.

Reaction conditions :

pH = 5.0

Temperature = 80 °C

$10^4[Arg]_T$ (mol dm^{-3})	$10^2[ninhydrin]_T$ (mol dm^{-3})	10^5k_{obs} (s^{-1})	10^5k_{cal} (s^{-1})
1.0	0.5	4.3	
1.5	0.5	4.1	
2.0	0.5	4.7	
2.5	0.5	4.3	
3.0	0.5	4.8	
3.5	0.5	4.9	
1.0	0.5	4.3	4.4
1.0	1.0	8.8	8.3
1.0	1.5	12.1	11.8
1.0	2.0	16.3	14.9
1.0	2.5	18.1	17.7
1.0	3.0	19.5	20.2
1.0	3.5	20.4	22.5
1.0	4.0	21.4	24.7

TABLE 3.15

Effect of [arginine] and [ninhydrin] on the *pseudo*-first-order rate constants (k_ψ) for the reaction of arginine with ninhydrin.

Reaction conditions :

$[CTAB]_T$ = 1.0×10^{-2} mol dm^{-3}

pH = 5.0

Temperature = 80 °C

$10^4[Arg]_T$ (mol dm^{-3})	$10^2[ninhydrin]_T$ (mol dm^{-3})	$10^5 k_\psi$ (s^{-1})
1.0	0.5	10.0
1.5	0.5	10.9
2.0	0.5	10.3
2.5	0.5	10.8
3.0	0.5	10.1
3.5	0.5	10.8
1.0	0.5	10.0
1.0	1.0	20.0
1.0	1.5	30.1
1.0	2.0	39.3
1.0	2.5	43.5
1.0	3.0	48.4
1.0	3.5	52.3
1.0	4.0	54.8

(when required) and pH (5.0) at 80 °C. The results are presented in Tables 3.5-3.15 and shown graphically in Figs. 3.7-3.12.

The plots k_{obs} and k_{ψ} versus [ninhydrin] were non-linear (except methionine) whereas log k_{obs} and log k_{ψ} vs. log [ninhydrin] plots were linear with slopes less than unity, this shows fractional order in [ninhydrin].

Dependence of the Reaction Rate on Surfactant Concentration

The dependence of the observed first-order rate constants on [CTAB] was studied by undertaking a number of kinetic experiments at different surfactant concentrations at constant $[ninhydrin]_T$ and $[amino\ acid]_T$ at pH = 5.0 and temperature 80 °C. The resutls are given in Tables 3.16-3.20 and depicted graphically in Figs. 3.13-3.17 as rate constant (k_{ψ}) - surfactant concentration profiles.

The observed *pseudo*-first-order rate constants, k_{ψ}, typically increase with increasing concentration of CTAB and reach constant values. The effect of higher concentration was seen in case of alanine and arginine where a decreasing effect was observed; the effect was not explored with threonine, tyrosine and glutamic acid as extensive foaming occurred beyond $[CTAB]_T > 80 \times 10^{-3}$ mol dm^{-3}.

Solvent Effect on the Reaction Rate

The effect of presence of organic solvents, viz. methyl cellosolve (MCS), 1-propanol (C_3OH), dimethyl sulfoxide (DMSO) and acetonitrile (AN)

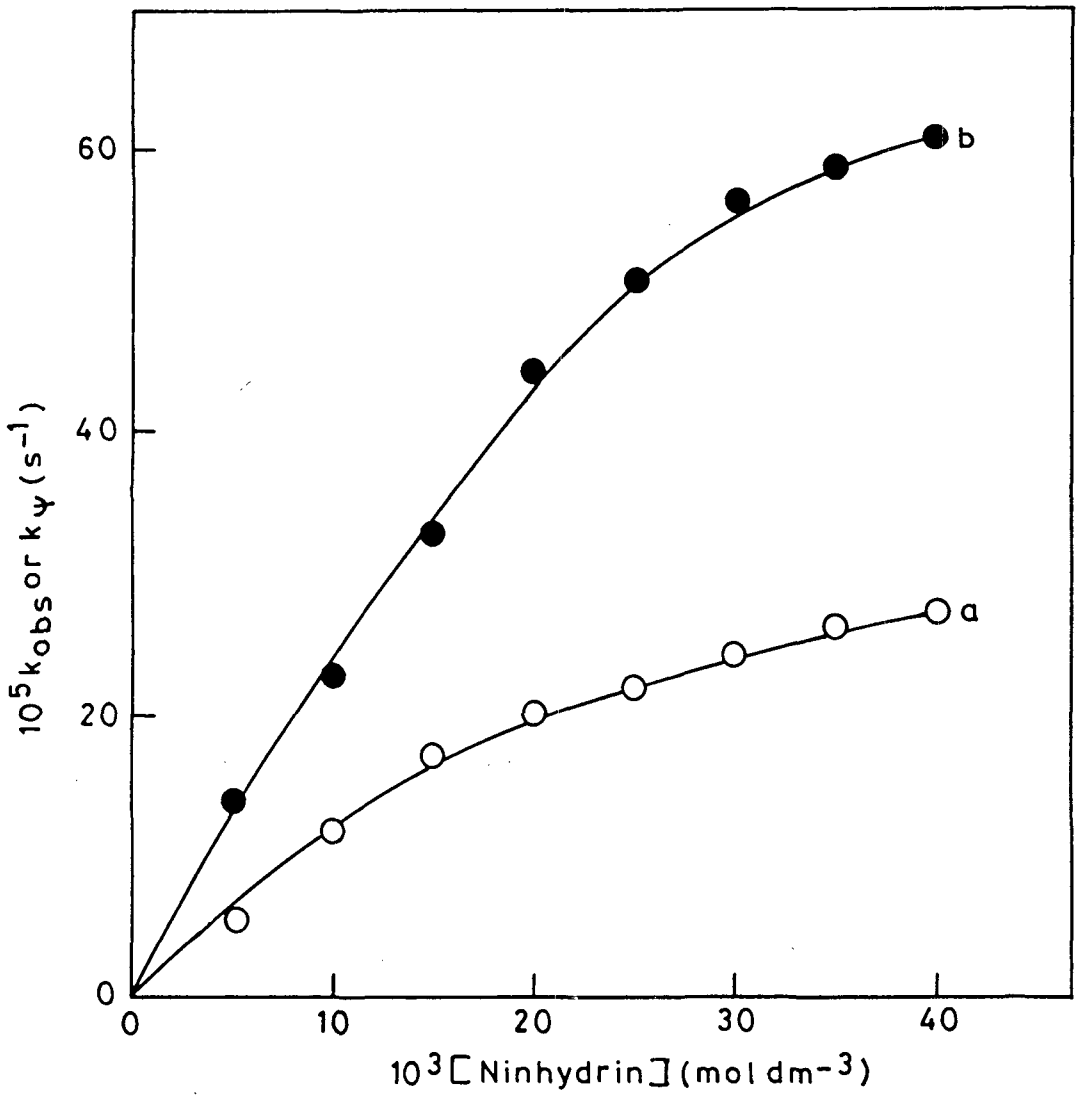


Fig. 3.7: Effect of [ninhydrin] on the reaction rate of alanine with ninhydrin in absence of CTAB (a), and in presence of 1.0×10^{-2} mol dm^{-3} CTAB (b). *Reaction conditions*: $[alanine]_T = 3.0 \times 10^{-4}$ mol dm^{-3}, pH = 5.0, temp. = 80 °C.

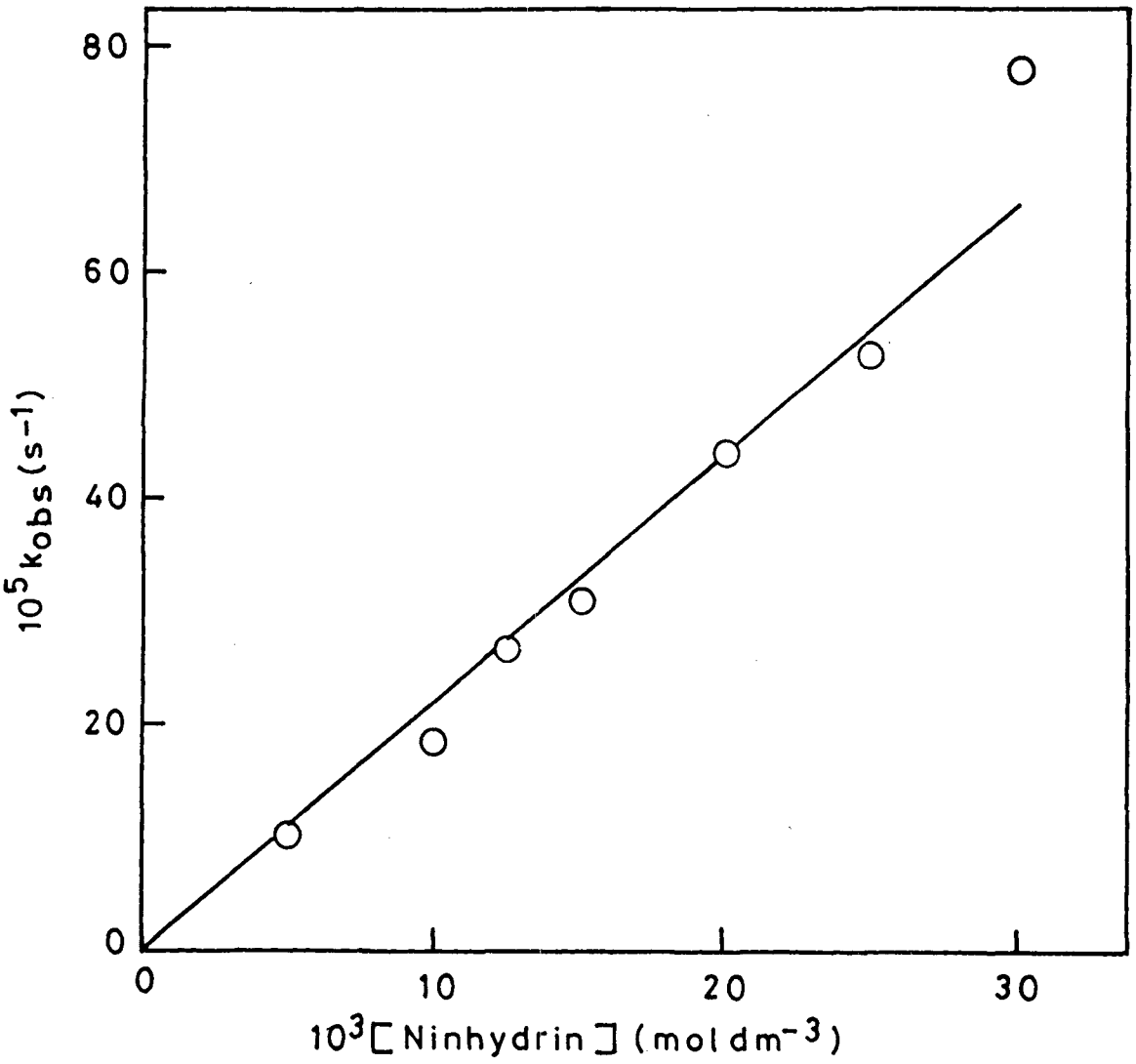


Fig. 3.8: Effect of [ninhydrin] on the reaction rate of methionine with ninhydrin. *Reaction conditions* : $[methionine]_T$ = 3.0 x 10^{-4} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

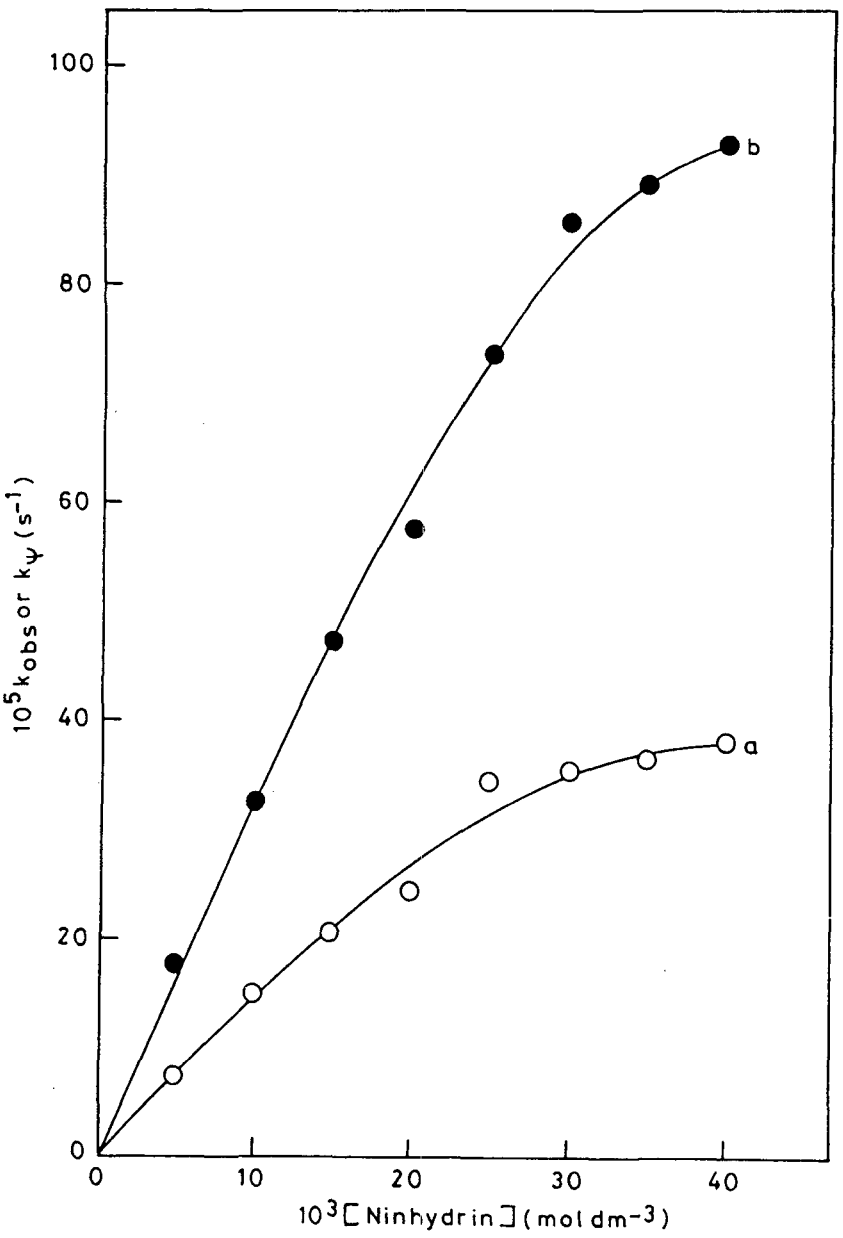


Fig. 3.9: Effect of [ninhydrin] on the reaction rate of threonine with ninhydrin in absence of CTAB (a), and in presence of 2.0×10^{-2} mol dm^{-3} CTAB (b). *Reaction conditions* : $[threonine]_T$ = 2.0×10^{-4} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

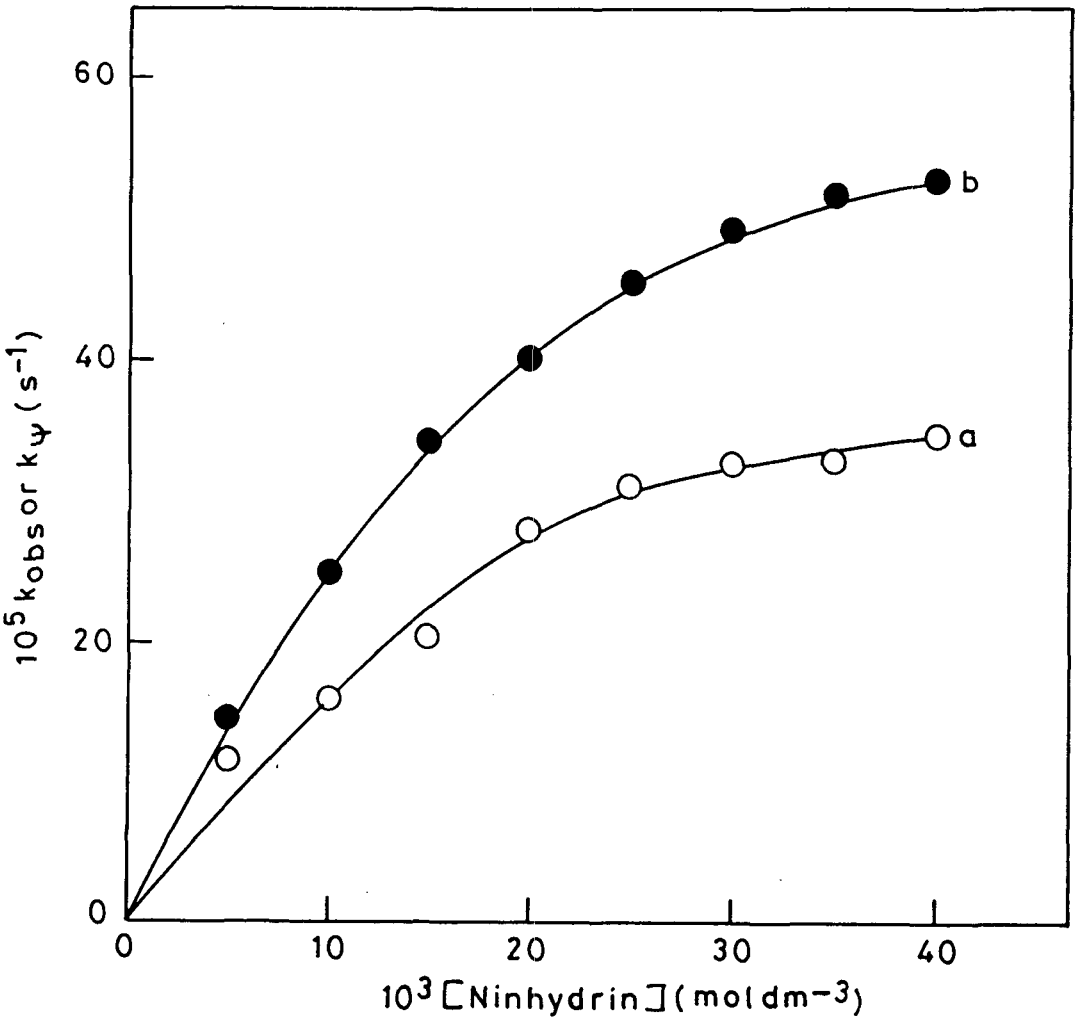


Fig. 3.10: Effect of [ninhydrin] on the reaction rate of tyrosine with ninhydrin in absence of CTAB (a), and in presence of 2.0×10^{-2} mol dm^{-3} CTAB (b). *Reaction conditions* : $[tyrosine]_T$ = 1.0×10^{-4} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

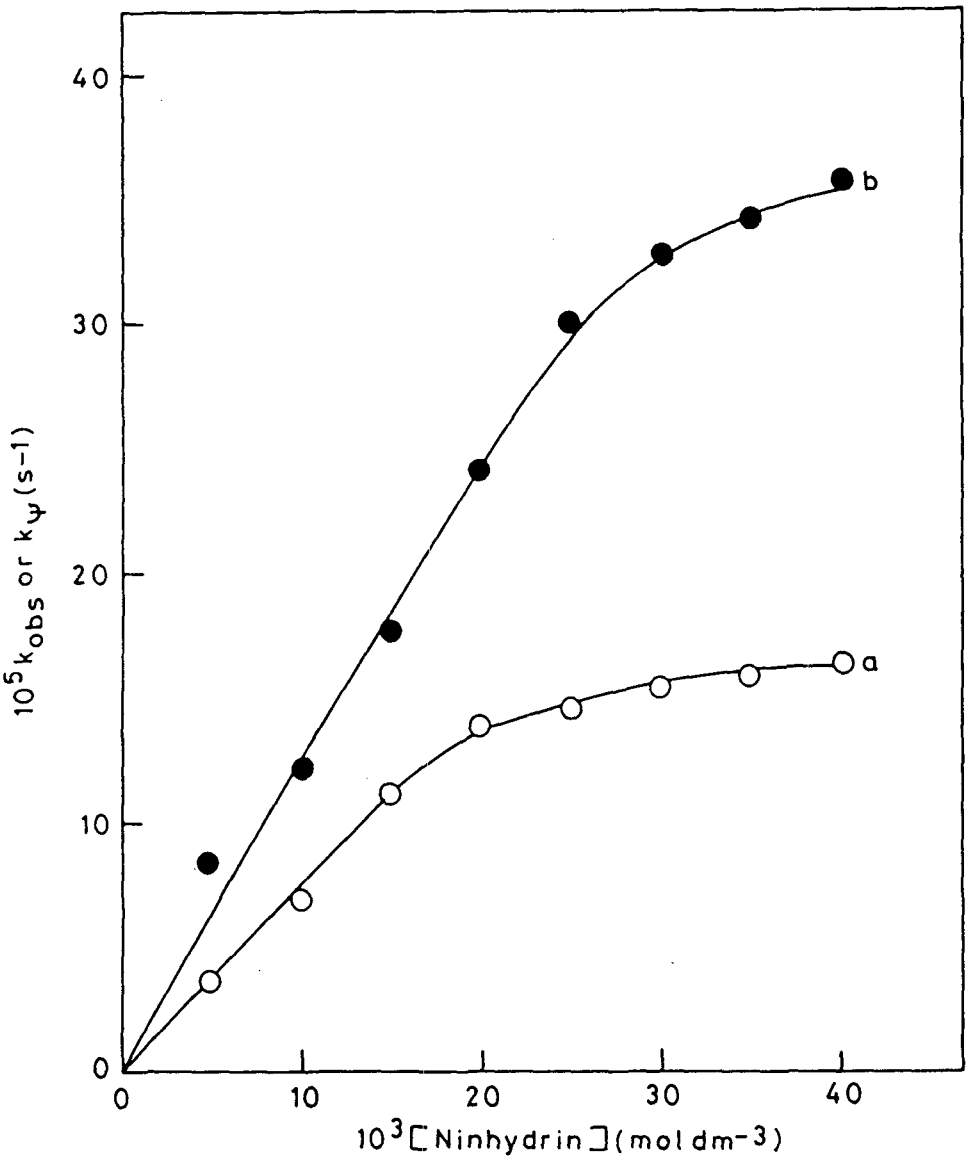


Fig. 3.11: Effect of [ninhydrin] on the reaction rate of glutamic acid with ninhydrin in absence of CTAB (a), and in presence of 2.0 x 10^{-2} mol dm^{-3} CTAB (b). *Reaction conditions* : [glutamic acid]$_T$ = 1.0 x 10^{-4} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

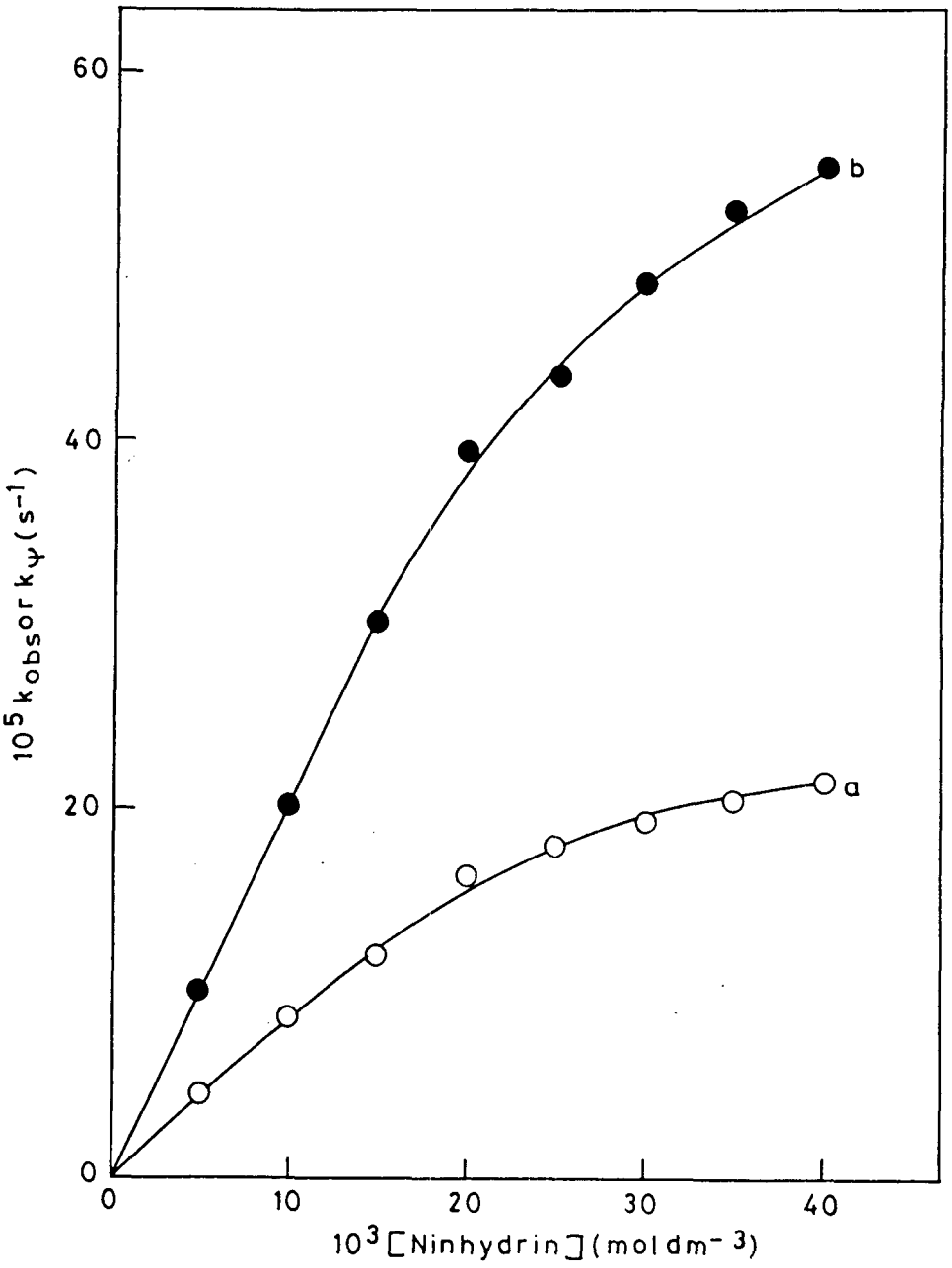


Fig. 3.12: Effect of [ninhydrin] on the reaction rate of arginine with ninhydrin in absence of CTAB (a), and in presence of 1.0 x 10^{-2} mol dm^{-3} CTAB (b). *Reaction conditions* : $[arginine]_T$ = 1.0 x 10^{-4} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

TABLE 3.16

Effect of [CTAB] on the *pseudo*-first-order rate constants (k_ψ) for the reaction of alanine with ninhydrin.

Reaction conditions :

$[alanine]_T$ = 3.0×10^{-4} mol dm^{-3}

$[ninhydrin]_T$ = 5.0×10^{-3} mol dm^{-3}

pH = 5.0

Temperature = 80 °C

$10^3[CTAB]_T$ (mol dm^{-3})	$10^5 k_\psi$ (s^{-1})	$10^5 k_{\psi cal}$ [a] (s^{-1})	$(k_\psi - k_{\psi cal})/k_\psi$
0	5.4	5.4	0
5	8.6	8.2	+0.04
10	13.4	12.9	+0.03
15	13.5	12.8	+0.05
20	13.6	12.9	+0.05
30	13.1	12.9	+0.01
40	13.6	13.5	0
50	13.0	12.8	+0.01
60	13.8	16.0	–0.15
70	13.4	12.7	+0.05
80	12.8	–	–
100	10.5	–	–

[a]calculated from eq. (3.9).

TABLE 3.17

Effect of [CTAB] on the *pseudo*-first-order rate constants (k_ψ) for the reaction of threonine with ninhydrin.

Reaction conditions :

$[threonine]_T$ = 2.0×10^{-4} mol dm^{-3}

$[ninhydrin]_T$ = 5.0×10^{-3} mol dm^{-3}

pH = 5.0

Temperature = 80 °C

$10^3[CTAB]_T$ (mol dm^{-3})	$10^5 k_\psi$ (s^{-1})	$10^5 k_{\psi cal}$[a] (s^{-1})	$(k_\psi - k_{\psi cal})/k_\psi$
0	7.8	7.8	0
5	10.9	10.4	+0.04
10	13.3	12.2	+0.08
15	15.0	13.8	+0.08
20	17.4	16.3	+0.06
30	18.3	17.5	+0.04
40	18.4	17.6	+0.04
50	19.0	18.4	+0.03
60	19.5	19.1	+0.02
70	20.3	19.5	+0.04
80	20.8	20.7	+0.04

[a]calculated from eq. (3.9).

TABLE 3.18

Effect of [CTAB] on the *pseudo*-first-order rate constants (k_ψ) for the reaction of tyrosine with ninhydrin.

Reaction conditions :

$[tyrosine]_T$ = 1.0×10^{-4} mol dm^{-3}

$[ninhydrin]_T$ = 5.0×10^{-3} mol dm^{-3}

pH = 5.0

Temperature = 80 °C

$10^3[CTAB]_T$ (mol dm^{-3})	$10^5 k_\psi$ (s^{-1})	$10^5 k_{\psi cal}$[a] (s^{-1})	$(k_\psi - k_{\psi cal})/k_\psi$
0	11.5	11.5	0
5	12.0	11.7	+0.02
10	13.1	12.9	+0.02
15	13.9	13.8	+0.01
20	14.3	14.3	0
30	14.8	15.0	−0.01
40	15.1	14.9	+0.01
50	15.4	15.3	+0.01
60	16.0	15.6	+0.02
70	16.3	16.1	+0.01
80	16.8	16.4	+0.02

[a]calculated from eq. (3.9).

TABLE 3.19

Effect of [CTAB] on the *pseudo*-first-order rate constants (k_ψ) for the reaction of glutamic acid with ninhydrin.

Reaction conditions :

$[\text{glutamic acid}]_T$ = 1.0×10^{-4} mol dm^{-3}

$[\text{ninhydrin}]_T$ = 5.0×10^{-3} mol dm^{-3}

pH = 5.0

Temperature = 80 °C

$10^3[CTAB]_T$ (mol dm^{-3})	$10^5 k_\psi$ (s^{-1})	$10^5 k_{\psi cal}$ [a] (s^{-1})	$(k_\psi - k_{\psi cal})/k_\psi$
0	3.5	3.5	0
5	5.3	5.0	+0.05
10	6.5	6.0	+0.07
15	7.1	6.8	+0.04
20	8.3	8.1	+0.02
30	8.6	8.1	−0.05
40	8.9	8.6	+0.03
50	9.2	9.8	−0.06
60	9.5	9.0	+0.05
80	9.8	9.4	+0.04

[a]calculated from eq. (3.9).

TABLE 3.20

Effect of [CTAB] on the *pseudo*-first-order rate constants (k_ψ) for the reaction of arginine with ninhydrin.

Reaction conditions :

$[arginine]_T$ = 1.0×10^{-4} mol dm^{-3}

$[ninhydrin]_T$ = 5.0×10^{-3} mol dm^{-3}

pH = 5.0

Temperature = 80 °C

$10^3[CTAB]_T$ (mol dm^{-3})	$10^5 k_\psi$ (s^{-1})	$10^5 k_{\psi cal}$[a] (s^{-1})	$(k_\psi - k_{\psi cal})/k_\psi$
0	4.3	4.3	0
3	6.8	6.5	+0.04
5	8.2	7.6	+0.07
10	10.0	9.3	+0.07
15	9.8	9.1	+0.07
20	9.5	8.9	+0.06
30	9.8	9.7	+0.01
40	9.9	9.1	+0.08
50	9.2	9.1	+0.01
60	8.4	8.0	+0.04
70	7.3	6.8	+0.06
80	6.6	6.1	+0.08
100	6.1	5.9	+0.03

[a]calculated from eq. (3.9).

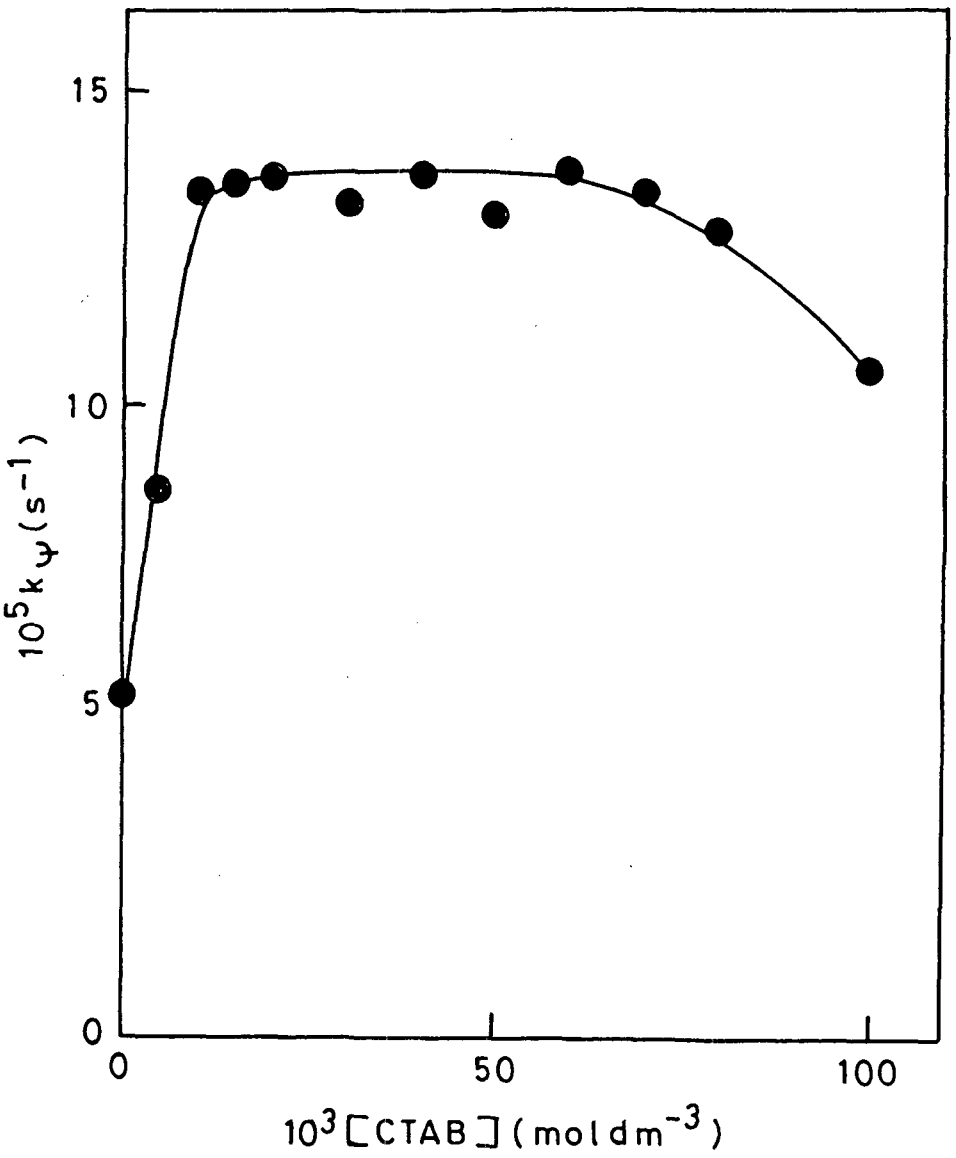


Fig. 3.13: Effect of [CTAB] on the reaction rate of alanine with ninhydrin. *Reaction conditions*: $[alanine]_T$ = 3.0 x 10^{-4} mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

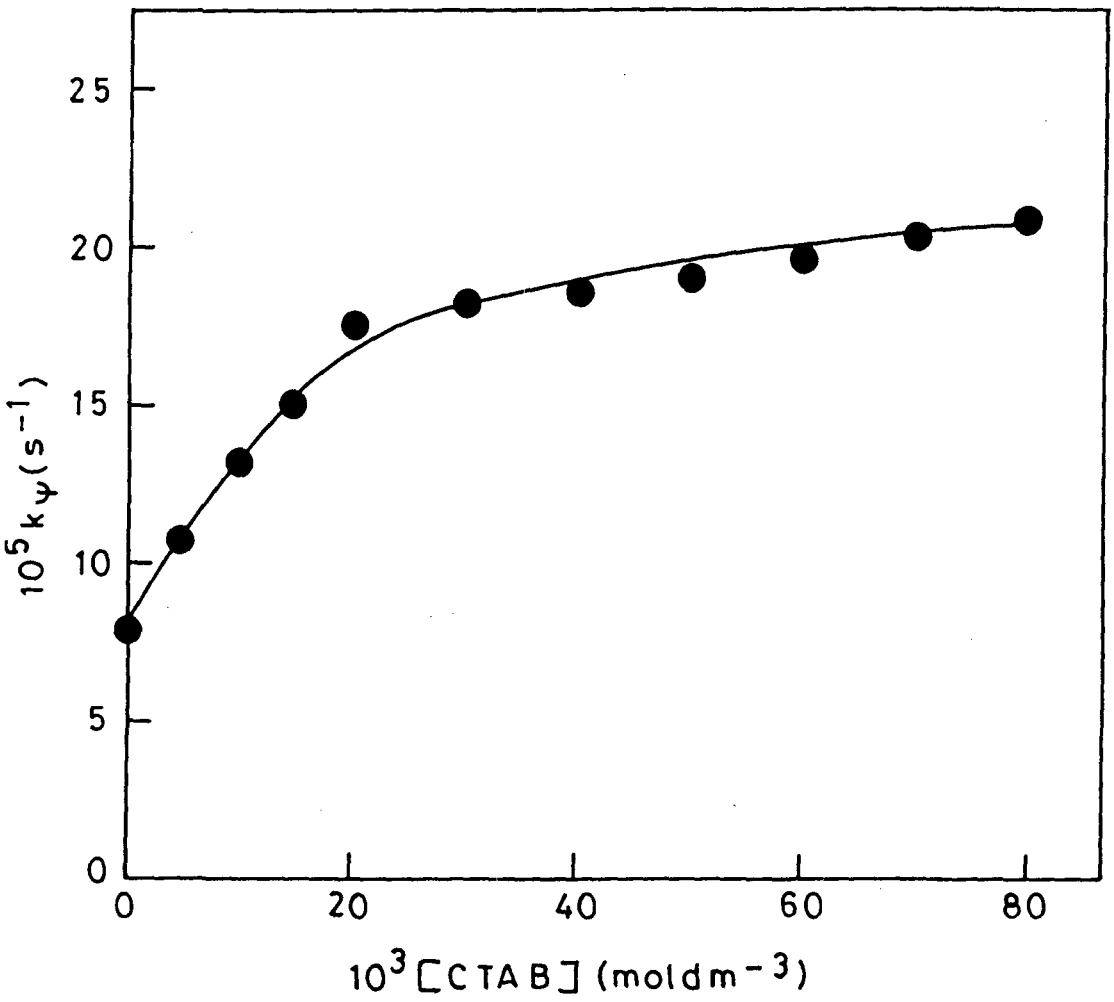


Fig. 3.14: Effect of [CTAB] on the reaction rate of threonine with ninhydrin. *Reaction conditions*: $[threonine]_T$ = 2.0 x 10^{-4} mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

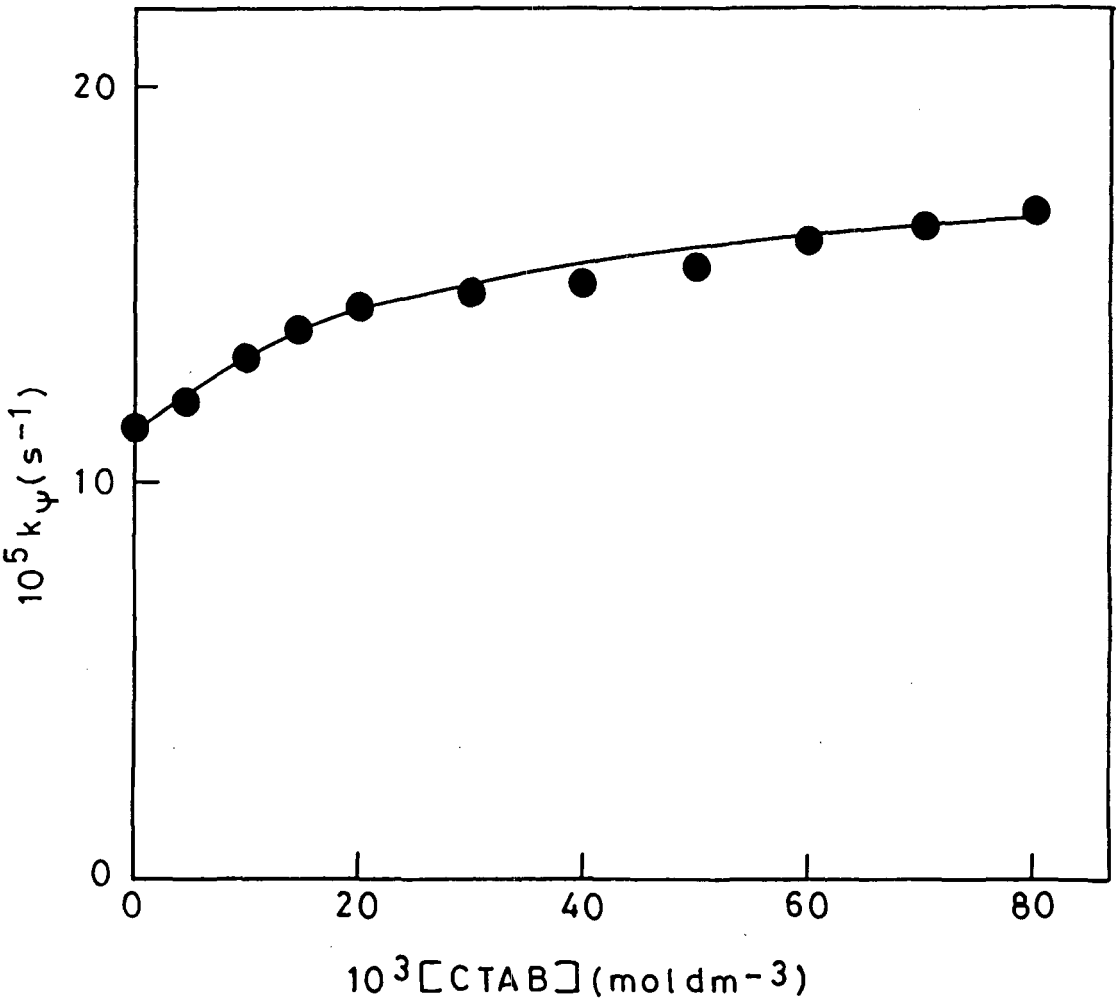


Fig. 3.15: Effect of [CTAB] on the reaction rate of tyrosine with ninhydrin. *Reaction conditions* : $[tyrosine]_T$ = 1.0 x 10^{-4} mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

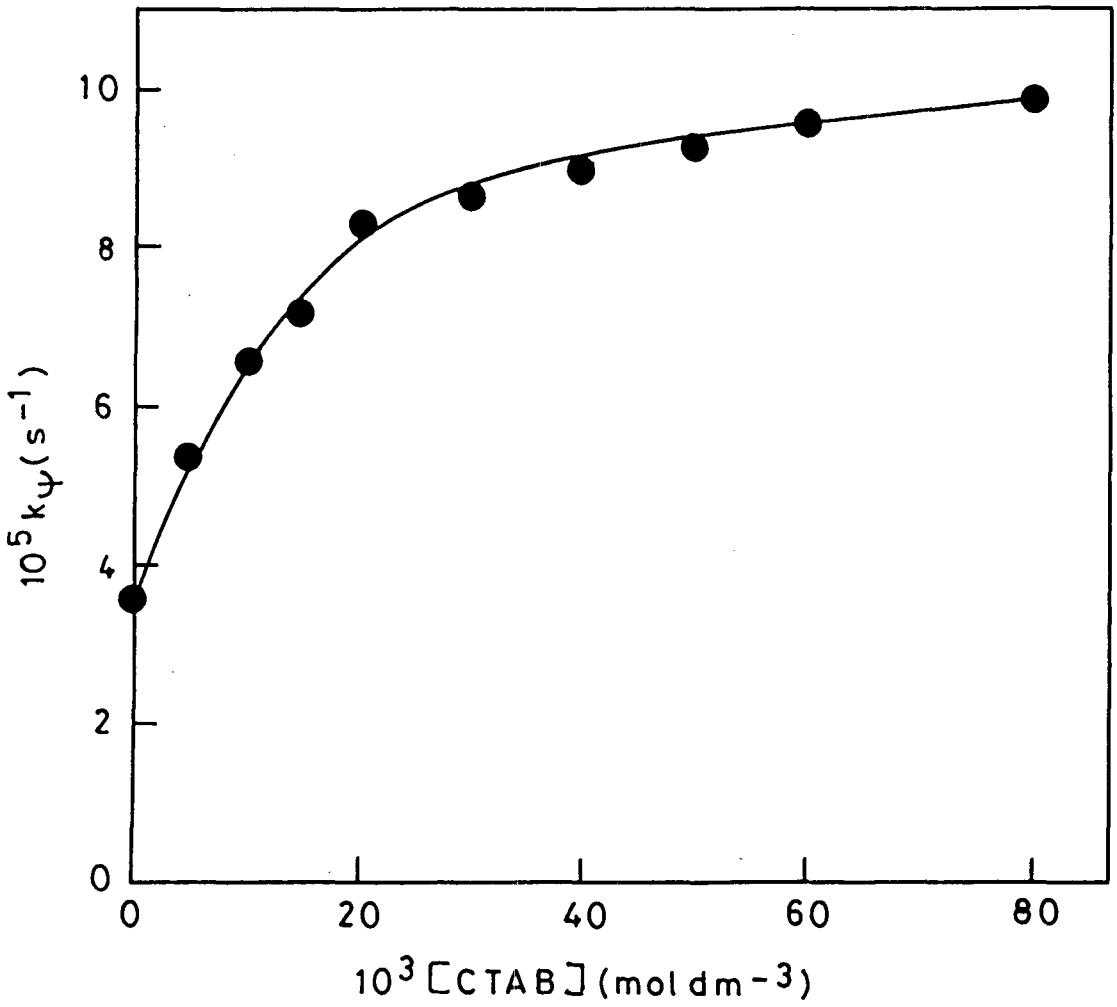


Fig. 3.16: Effect of [CTAB] on the reaction rate of glutamic acid with ninhydrin. *Reaction conditions* : [glutamic acid]$_T$ = 1.0 x 10^{-4} mol dm^{-3}, [ninhydrin]$_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

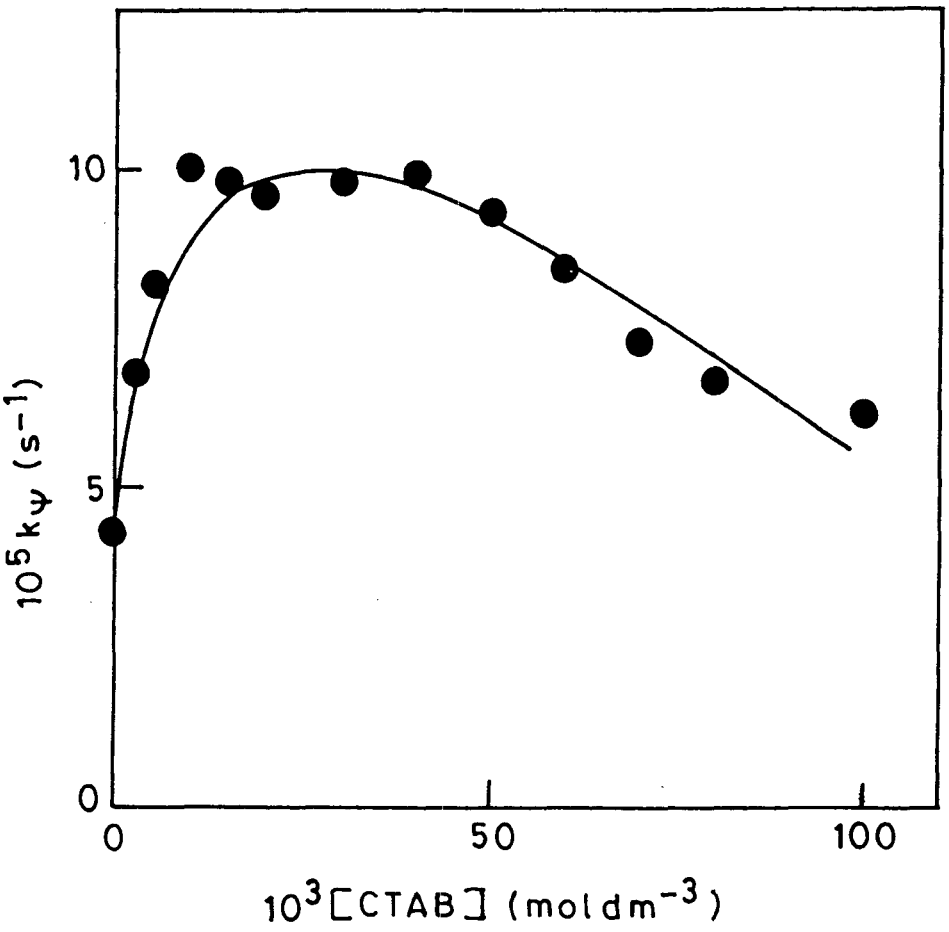


Fig. 3.17: Effect of [CTAB] on the reaction rate of arginine with ninhydrin. *Reaction conditions* : $[arginine]_T$ = 1.0 x 10^{-4} mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

on the rate of purple-colour formation was also seen at fixed $[\text{amino acid}]_T$, $[\text{ninhydrin}]_T$, pH (5.0) and temeprature (80 °C) (Tables 3.21-3.26, Figs. 3.18-3.23). The effect of DMSO on the rate of reaction was also observed in presence of CTAB. The results are recorded in Tables 3.21-3.26 and shown graphically in Figs. 3.24-3.28.

Dependence of the Reaction Rate on Temperature

The kinetic experiments were carried out at 70-90 °C (except in case of methionine where temperature range was at 75 - 95 °C) in the absence and presence of CTAB in order to derive activation parameters. The values of rate constants and relevent parameters are given in Tables 3.27-3.32. The data obtained were found to fit in the Eyring equation

$$k = (k_B T/h) \exp (\Delta S^{\#}/R) \exp (-\Delta H^{\#}/RT) \qquad (3.2)$$

(k_B, h and R are, respectively, Boltzmann, Planck and gas constants).

TABLE 3.21

Effect of organic solvents on the *pseudo*-first-order rate constants (k_{obs} or k_{ψ}) for the reaction of alanine with ninhydrin.

Reaction conditions :

$[alanine]_T$ = 3.0×10^{-4} mol dm^{-3}

$[ninhydrin]_T$ = 5.0×10^{-3} mol dm^{-3}

pH = 5.0

Temperature = 80 °C

% Solvent	$10^5 k_{obs}$ (s^{-1})				$10^5 k_{\psi}$ (s^{-1})
(v/v)	AN	DMSO	MCS	C_3OH	DMSO[a]
0	5.4	5.4	5.4	5.4	13.4
5	15.1	9.2	8.4	8.3	14.0
10	25.8	11.7	10.8	11.6	16.0
15	40.6	16.9	11.9	17.8	22.7
20	54.6	21.7	13.2	19.6	28.9
25	60.0	28.0	15.5	26.3	45.5
30	76.0	46.0	19.7	32.9	54.3
35	94.9	52.2	34.6	–	–
40	–	74.4	47.1	41.7	–

[a][CTAB] = 1.0×10^{-2} mol dm^{-3}.

TABLE 3.22

Effect of organic solvents on the *pseudo*-first-order rate constants (k_{obs}) for the reaction of methionine with ninhydrin.

Reaction conditions :

$[ninhydrin]_T$ = 5.0×10^{-3} mol dm^{-3}

pH = 5.0

Temperature = 80 °C

% Solvent	$10^5 k_{obs}$ (s^{-1})			
(v/v)	AN[a]	DMSO[a]	MCS[b]	C_3OH[b]
0	no reaction	no reaction	10.6	10.6
5	11.9	5.7	10.5	11.1
10	26.8	12.0	22.5	11.1
15	33.2	16.8	25.5	14.0
20	40.4	28.0	27.0	26.0
25	50.2	36.7	44.2	38.5
30	78.1	52.2	45.6	74.6
35	–	42.4	56.2	–
40	–	47.4	70.4	–

[a]$[methionine]_T = 1.0 \times 10^{-4}$ mol dm^{-3}.

[b]$[methionine]_T = 3.0 \times 10^{-4}$ mol dm^{-3}.

TABLE 3.23

Effect of organic solvents on the *pseudo*-first-order rate constants (k_{obs} or k_{ψ}) for the reaction of threonine with ninhydrin.

Reaction conditions :

$[threonine]_T$ = 2.0×10^{-4} mol dm^{-3}

$[ninhydrin]_T$ = 5.0×10^{-3} mol dm^{-3}

pH = 5.0

Temperature = 80 °C

% Solvent	$10^5 k_{obs}$ (s^{-1})				$10^5 k_{\psi}$ (s^{-1})
(v/v)	AN	DMSO	MCS	C_3OH	DMSO[a]
0	7.8	7.8	7.8	7.8	17.4
5	11.3	8.2	9.3	10.1	18.6
10	18.2	9.3	11.5	14.0	19.5
15	24.5	12.1	14.1	17.9	21.3
20	30.4	16.1	19.1	24.7	27.5
25	39.0	19.8	23.5	31.1	31.2
30	47.2	23.8	26.8	37.0	44.8
35	52.5	27.8	33.2	41.2	–
40	62.8	51.4	59.6	47.9	–

[a][CTAB] = 2.0×10^{-2} mol dm^{-3}.

TABLE 3.24

Effect of organic solvents on the *pseudo*-first-order rate constants (k_{obs} or k_{ψ}) for the reaction of tyrosine with ninhydrin.

Reaction conditions :

$[tyrosine]_T$ = 1.0×10^{-4} mol dm^{-3}

$[ninhydrin]_T$ = 5.0×10^{-3} mol dm^{-3}

pH = 5.0

Temperature = 80 °C

% Solvent	$10^5 k_{obs}$ (s^{-1})				$10^5 k_{\psi}$ (s^{-1})
(v/v)	AN	DMSO	MCS	C_3OH	DMSO[a]
0	11.5	11.5	11.5	11.5	14.3
5	17.1	11.9	15.2	13.2	16.3
10	19.8	12.5	19.4	17.3	17.2
15	27.1	13.2	23.1	21.2	19.4
20	35.2	17.1	29.2	27.2	22.3
25	43.7	22.5	37.1	35.9	27.0
30	53.1	27.2	49.7	44.1	34.2
35	60.0	31.6	70.0	49.9	41.5
40	75.1	37.4	100.3	57.2	48.0

[a][CTAB] = 2.0×10^{-2} mol dm^{-3}.

TABLE 3.25

Effect of organic solvents on the *pseudo*-first-order rate constants (k_{obs} or k_{ψ}) for the reaction of glutamic acid with ninhydrin.

Reaction conditions :

$[\text{glutamic acid}]_T$ = 1.0×10^{-4} mol dm^{-3}

$[\text{ninhydrin}]_T$ = 5.0×10^{-3} mol dm^{-3}

pH = 5.0

Temperature = 80 °C

% Solvent	$10^5 k_{obs}$ (s^{-1})				$10^5 k_{\psi}$ (s^{-1})
(v/v)	AN	DMSO	MCS	C_3OH	DMSO[a]
0	3.5	3.5	3.5	3.5	8.3
5	4.3	4.0	4.7	4.4	9.2
10	5.2	4.8	6.0	5.0	11.8
15	6.6	5.6	8.0	7.6	13.4
20	9.1	8.6	9.8	16.5	16.6
25	12.5	16.6	12.3	21.9	22.1
30	17.0	19.4	15.9	28.9	27.9
35	25.9	23.6	29.1	31.8	–
40	35.9	31.5	44.4	35.0	–

[a][CTAB] = 2.0×10^{-2} mol dm^{-3}.

TABLE 3.26

Effect of organic solvents on the *pseudo*-first-order rate constants (k_{obs} or k_{ψ}) for the reaction of arginine with ninhydrin.

Reaction conditions :

$[arginine]_T$ = 1.0×10^{-4} mol dm^{-3}

$[ninhydrin]_T$ = 5.0×10^{-3} mol dm^{-3}

pH = 5.0

Temperature = 80 °C

% Solvent	$10^5 k_{obs}$ (s^{-1})				$10^5 k_{\psi}$ (s^{-1})
(v/v)	AN	DMSO	MCS	C_3OH	DMSO[a]
0	4.3	4.3	4.3	4.3	10.0
5	9.8	8.3	10.0	8.4	12.1
10	14.6	13.5	15.9	13.1	18.5
15	20.1	21.1	20.7	17.7	25.7
20	27.7	30.0	27.3	22.6	36.3
25	32.3	35.3	32.3	26.0	40.1
30	39.6	45.1	37.7	29.6	50.7
35	56.8	53.3	43.1	33.6	60.0
40	82.6	68.7	48.7	37.7	75.3

[a][CTAB] = 1.0×10^{-2} mol dm^{-3}.

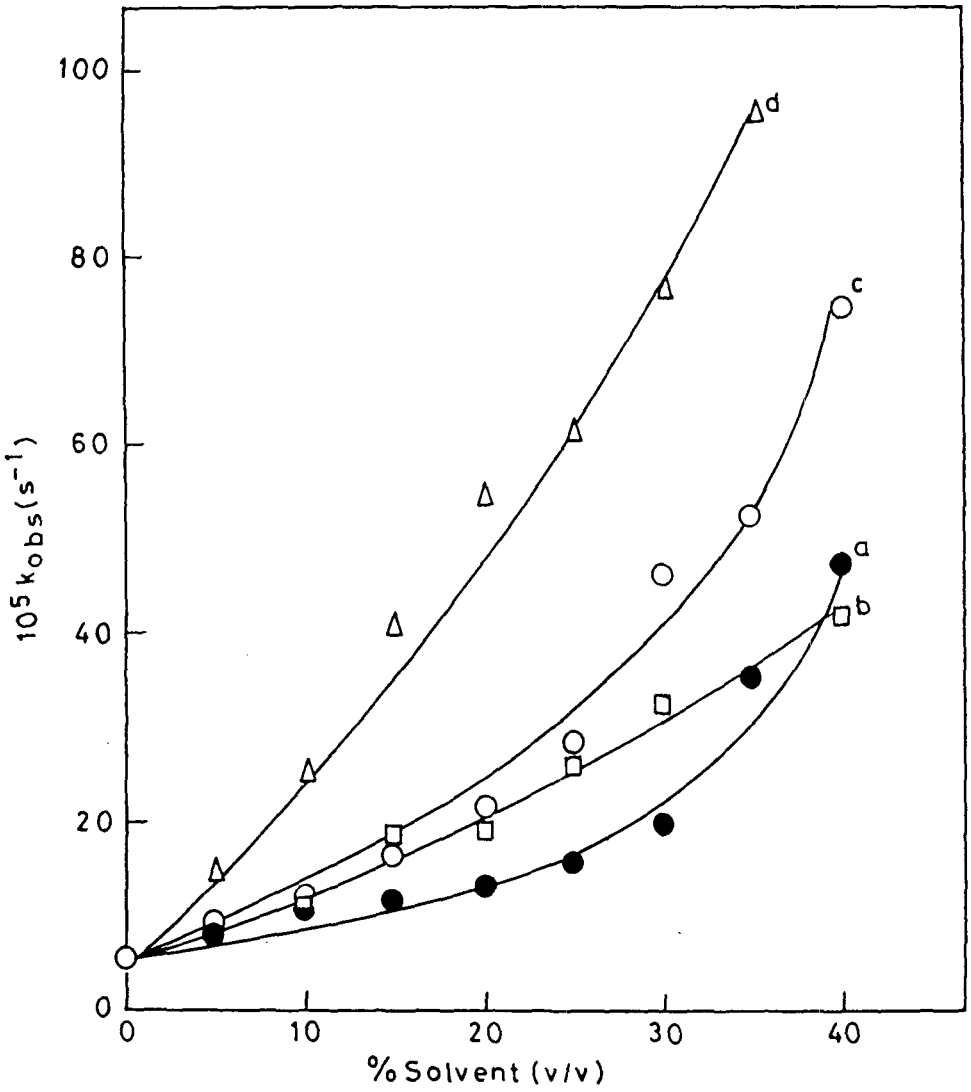


Fig. 3.18: Effect of methyl cellosolve (a), 1-propanol (b), dimethyl sulfoxide (c), and acetonitrile (d) on the reaction rate of alanine with ninhydrin. *Reaction conditions* : $[alanine]_T$ = 3.0 x 10^{-4} mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

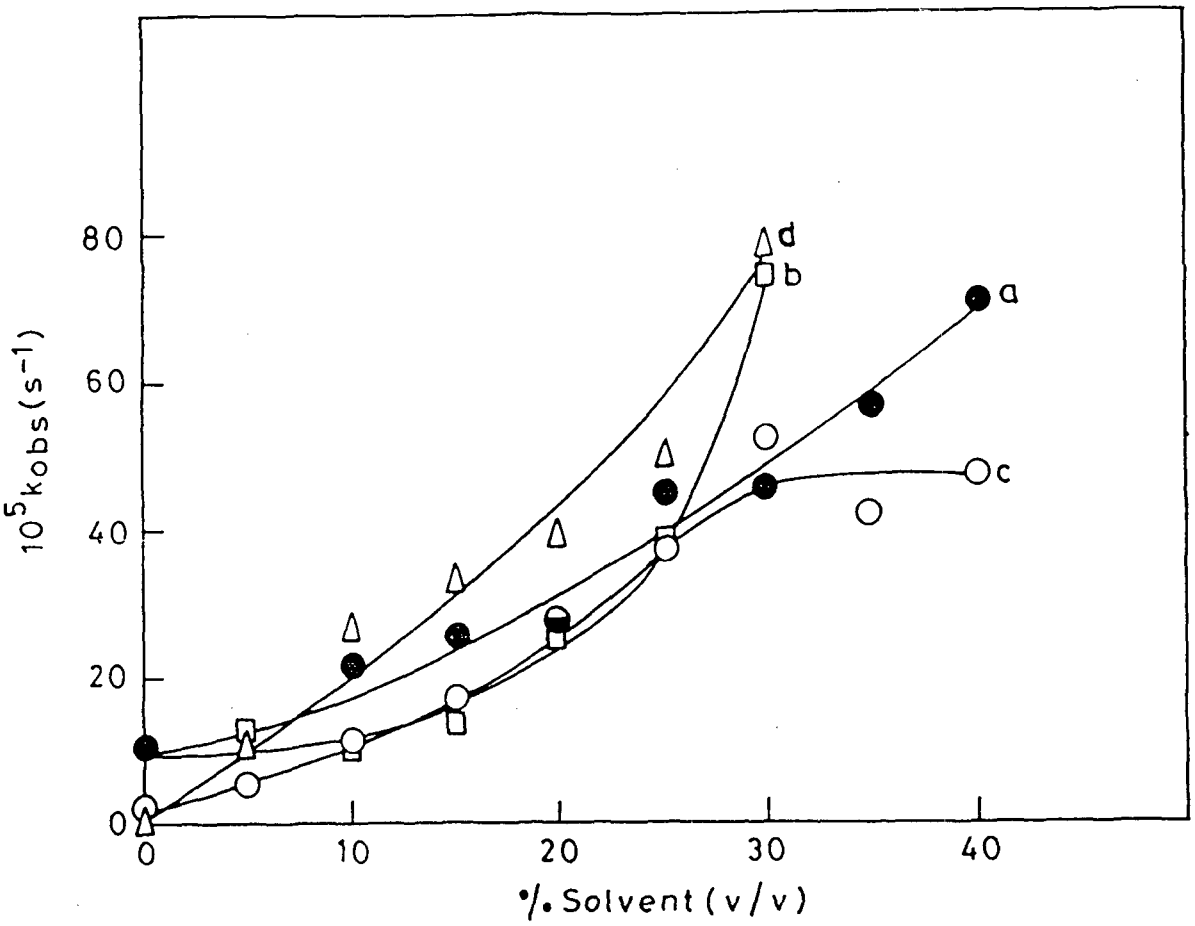


Fig. 3.19: Effect of methyl cellosolve (a), 1-propanol (b), dimethyl sulfoxide (c), and acetonitrile (d) on the reaction rate of methionine with ninhydrin. *Reaction conditions* : $[methionine]_T$ = 1.0 x 10^{-4} mol dm^{-3} (for acetonitrile and dimethylsulfoxide), $[methionine]_T$ = 3.0 x 10^{-4} mol dm^{-3} (for methyl cellosolve and 1-propanol), $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

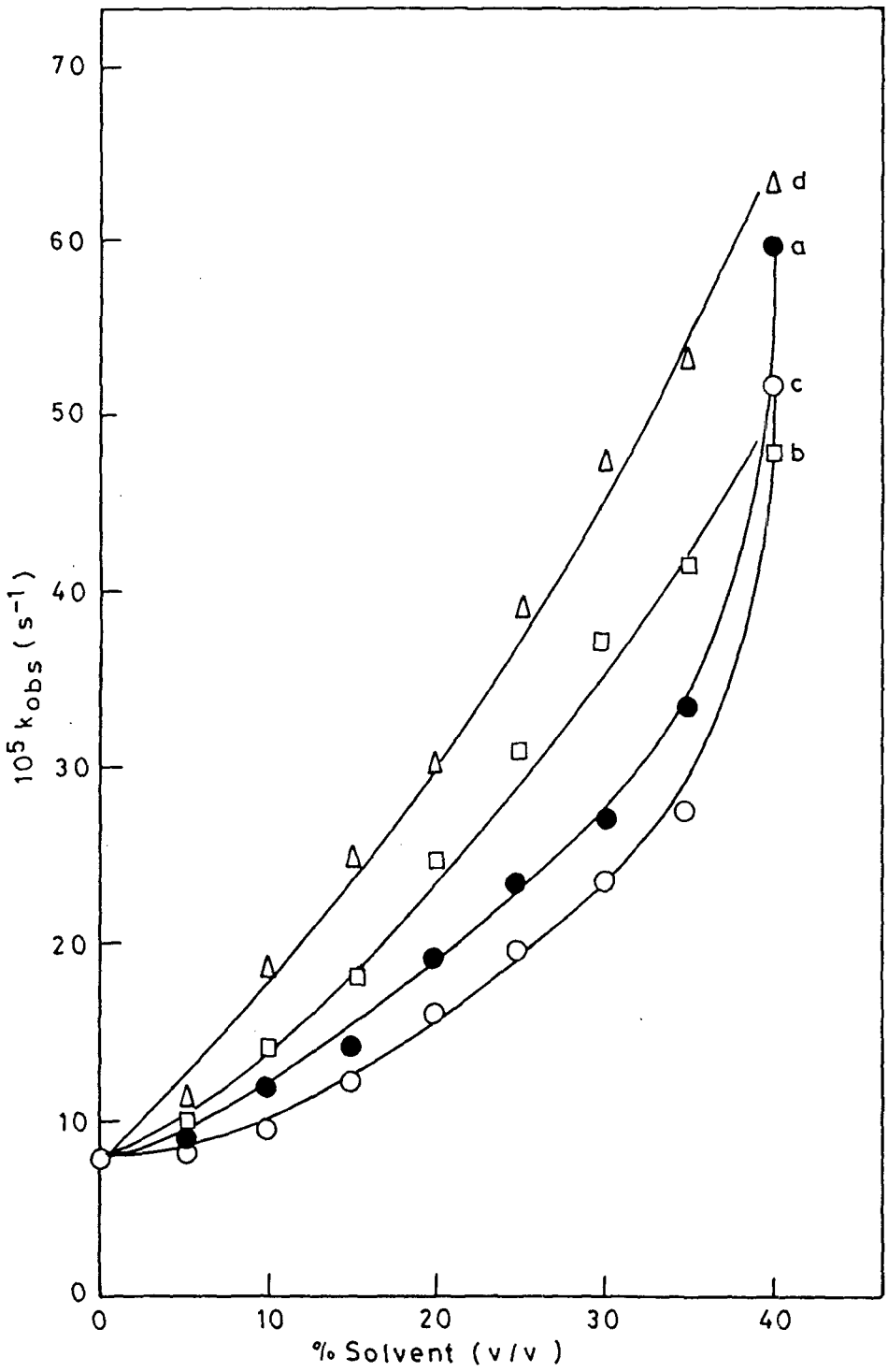


Fig. 3.20: Effect of methyl cellosolve (a), 1-propanol (b), dimethyl sulfoxide (c), and acetonitrile (d) on the reaction rate of threonine with ninhydrin. *Reaction conditions* : $[threonine]_T$ = 2.0 x 10^{-4} mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

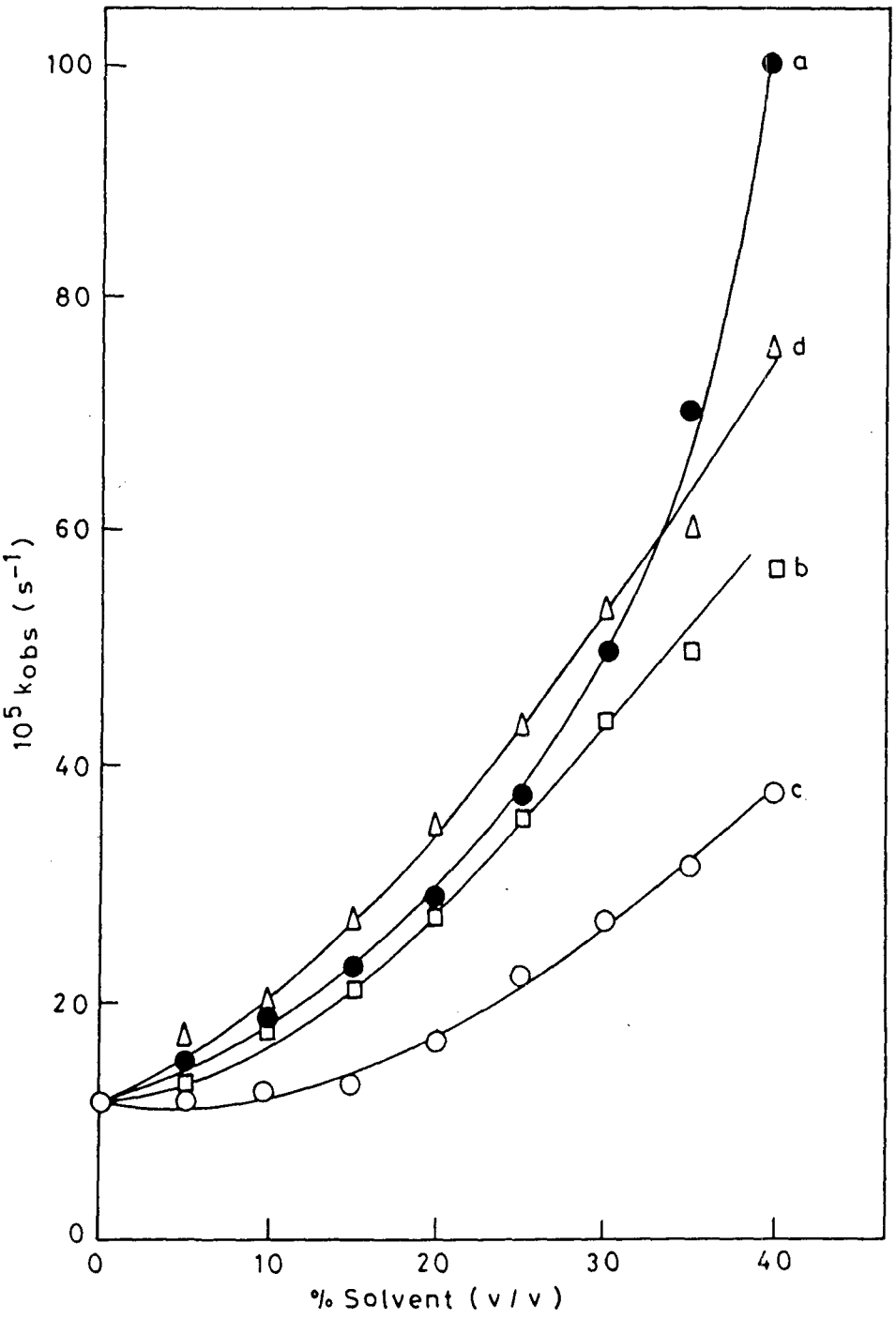


Fig. 3.21: Effect of methyl cellosolve (a), 1-propanol (b), dimethyl sulfoxide (c), and acetonitrile (d) on the reaction rate of tyrosine with ninhydrin. *Reaction conditions*: $[tyrosine]_T$ = 1.0 x 10^{-4} mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

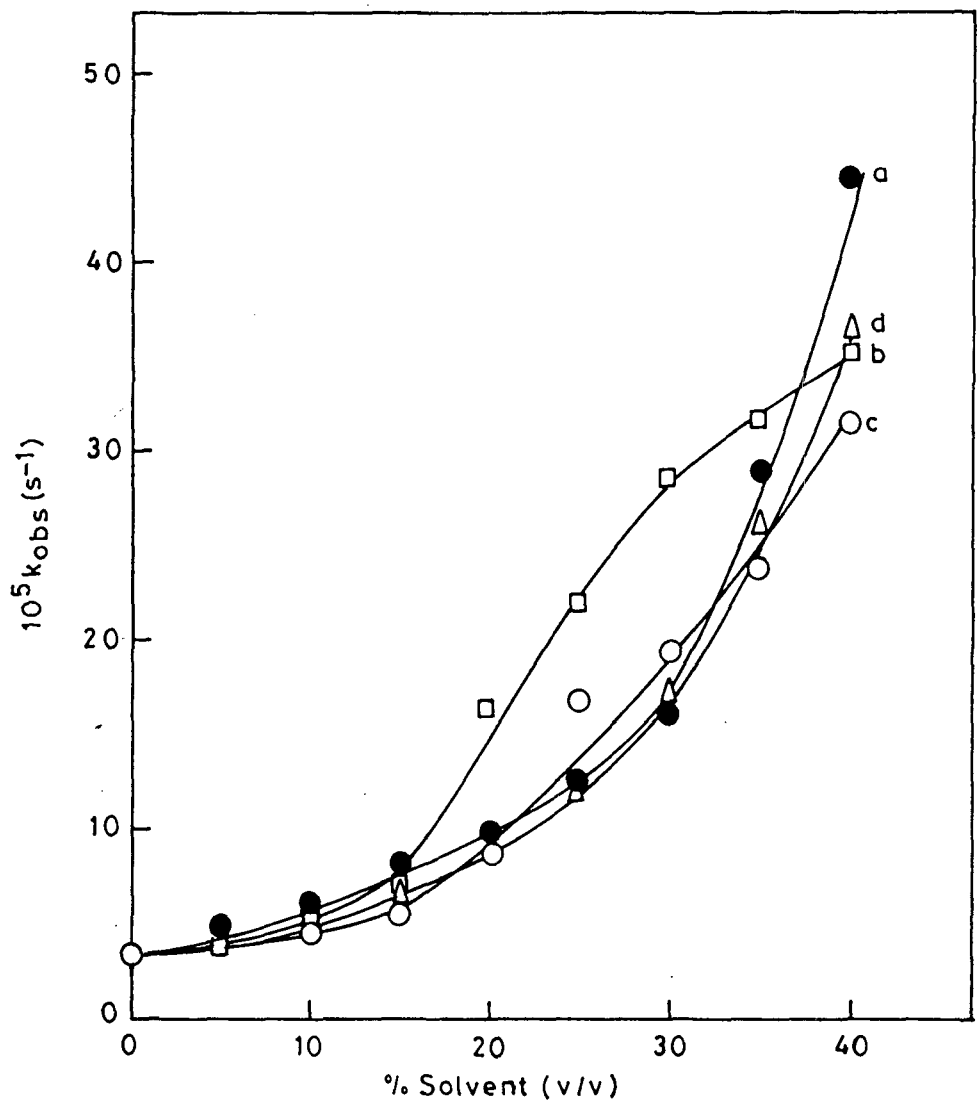


Fig. 3.22: Effect of methyl cellosolve (a), 1-propanol (b), dimethyl sulfoxide (c), and acetonitrile (d) on the reaction rate of glutamic acid with ninhydrin. *Reaction conditions* : $[\text{glutamic acid}]_T = 1.0 \times 10^{-4}$ mol dm^{-3}, $[\text{ninhydrin}]_T = 5.0 \times 10^{-3}$ mol dm^{-3}, pH = 5.0, temp. = 80 °C.

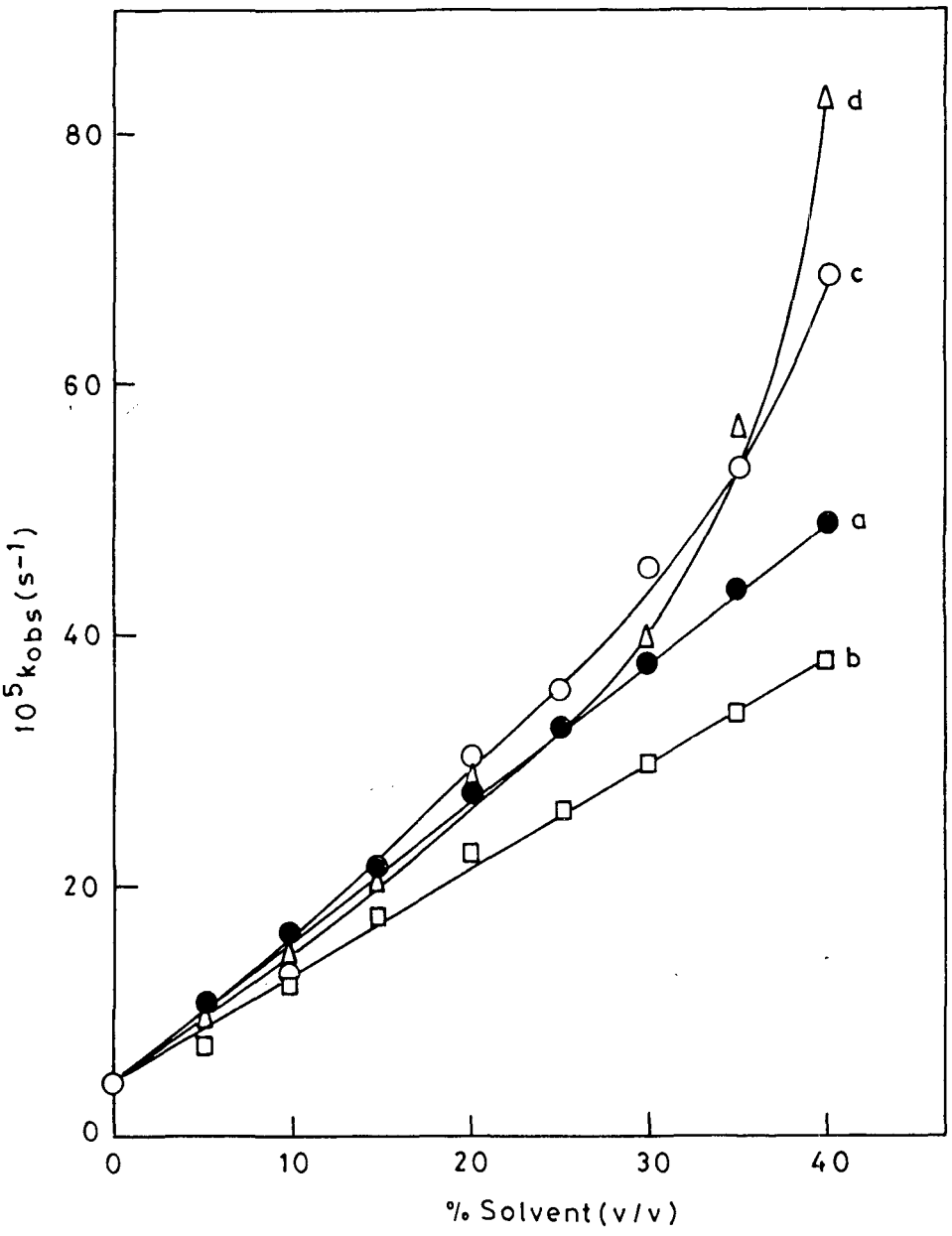


Fig. 3.23: Effect of methyl cellosolve (a), 1-propanol (b), dimethyl sulfoxide (c), and acetonitrile (d) on the reaction rate of arginine with ninhydrin. *Reaction conditions*: $[arginine]_T$ = 1.0 x 10^{-4} mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

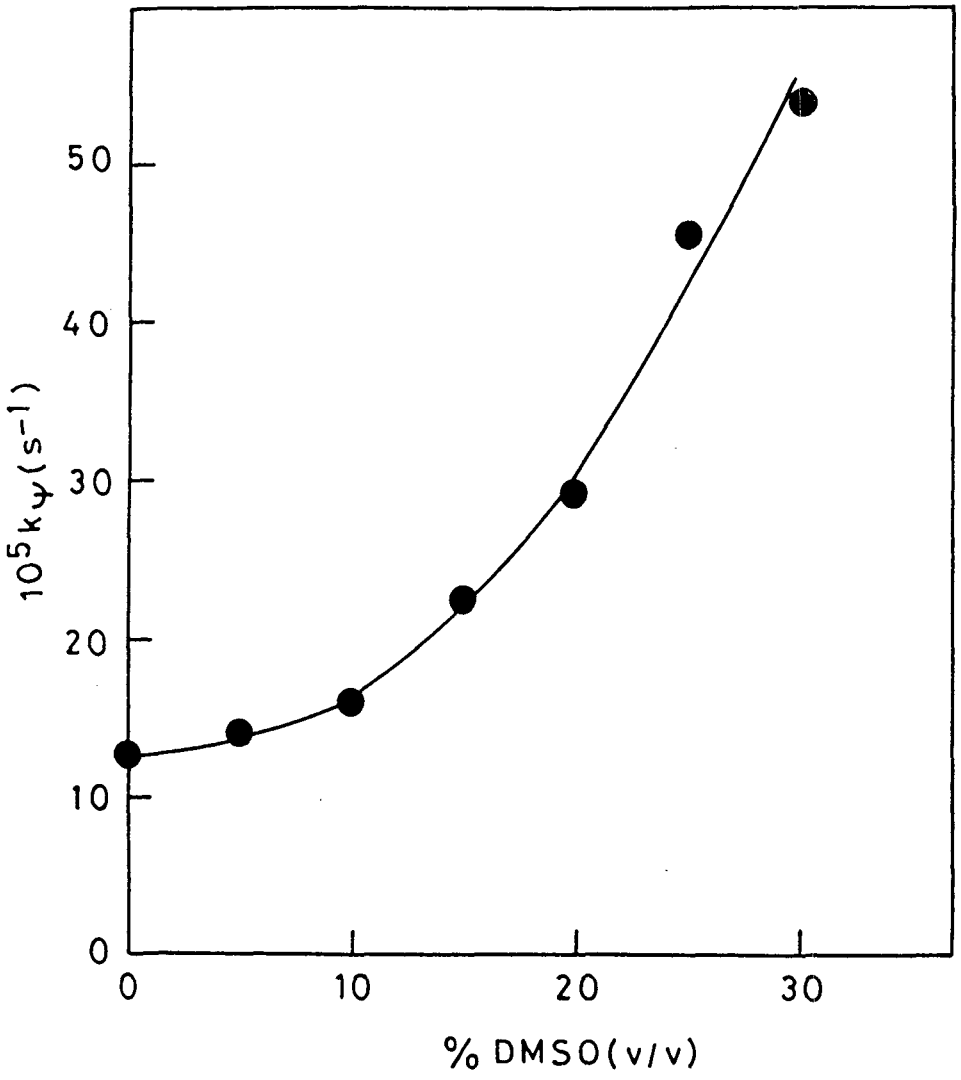


Fig. 3.24: Effect of DMSO on the reaction rate of alanine with ninhydrin in presence of CTAB. *Reaction conditions* : $[alanine]_T$ = 3.0 x 10^{-4} mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, $[CTAB]_T$ = 1.0 x 10^{-2} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

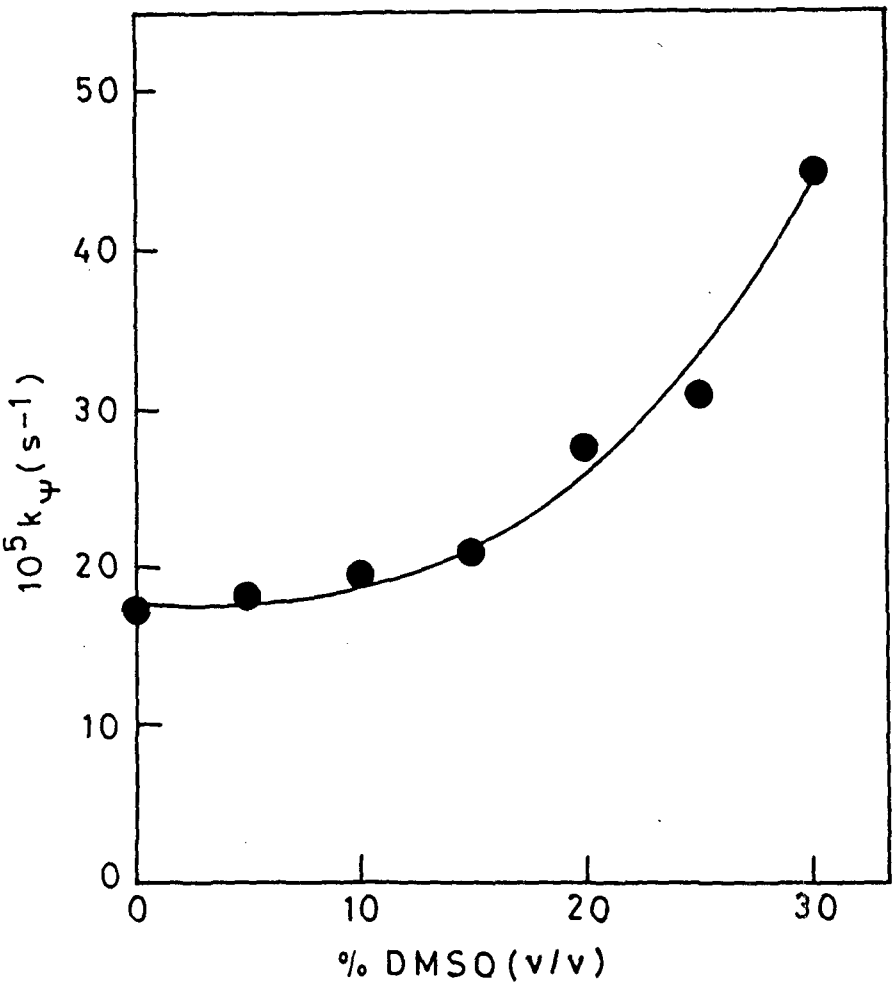


Fig. 3.25: Effect of DMSO on the reaction rate of threonine with ninhydrin in presence of CTAB. *Reaction conditions* : $[threonine]_T = 2.0 \times 10^{-4}$ mol dm^{-3}, $[ninhydrin]_T = 5.0 \times 10^{-3}$ mol dm^{-3}, $[CTAB]_T = 2.0 \times 10^{-2}$ mol dm^{-3}, pH = 5.0, temp. = 80 °C.

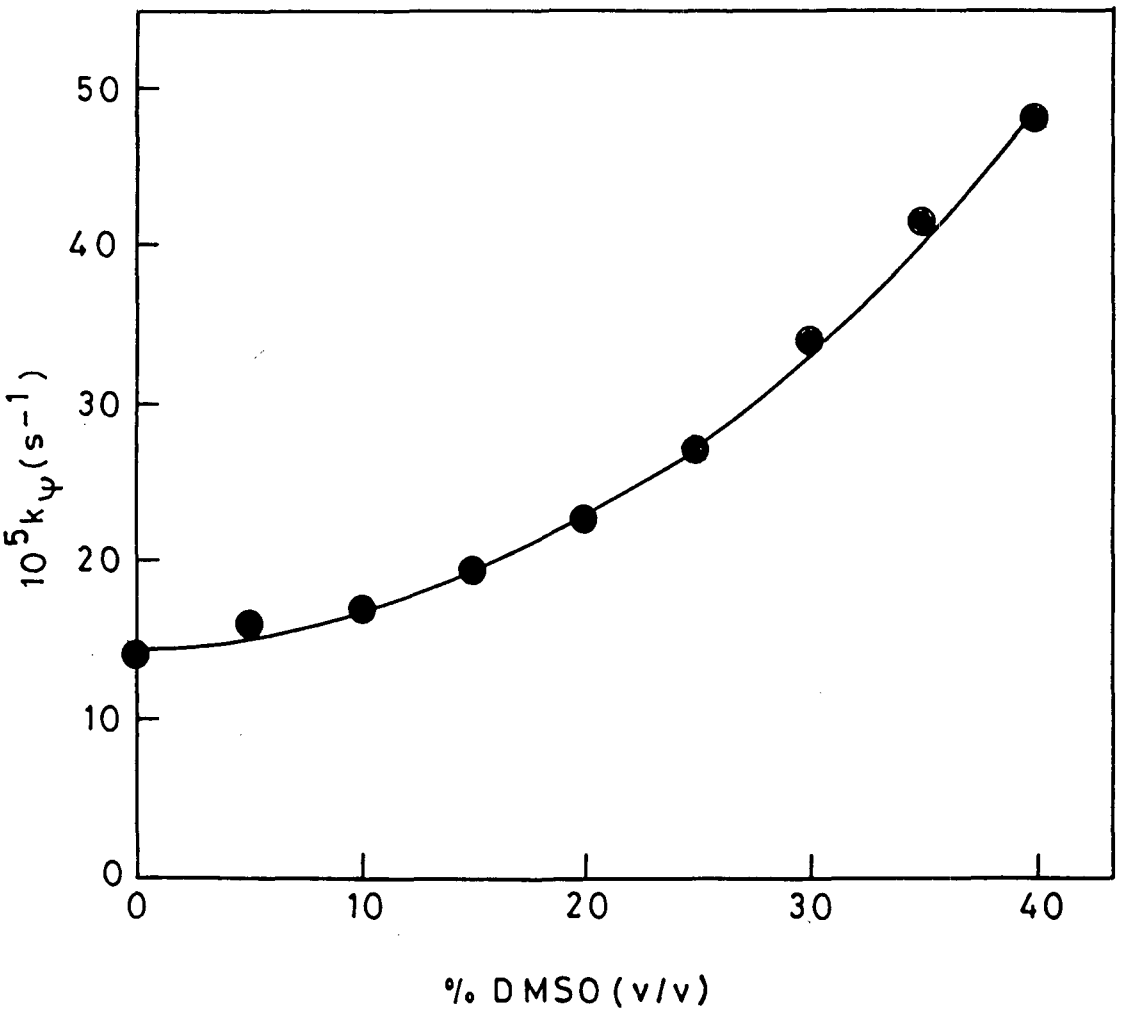


Fig. 3.26: Effect of DMSO on the reaction rate of tyrosine with ninhydrin in presence of CTAB. *Reaction conditions* : $[tyrosine]_T$ = 1.0 x 10^{-4} mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, $[CTAB]_T$ = 2.0 x 10^{-2} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

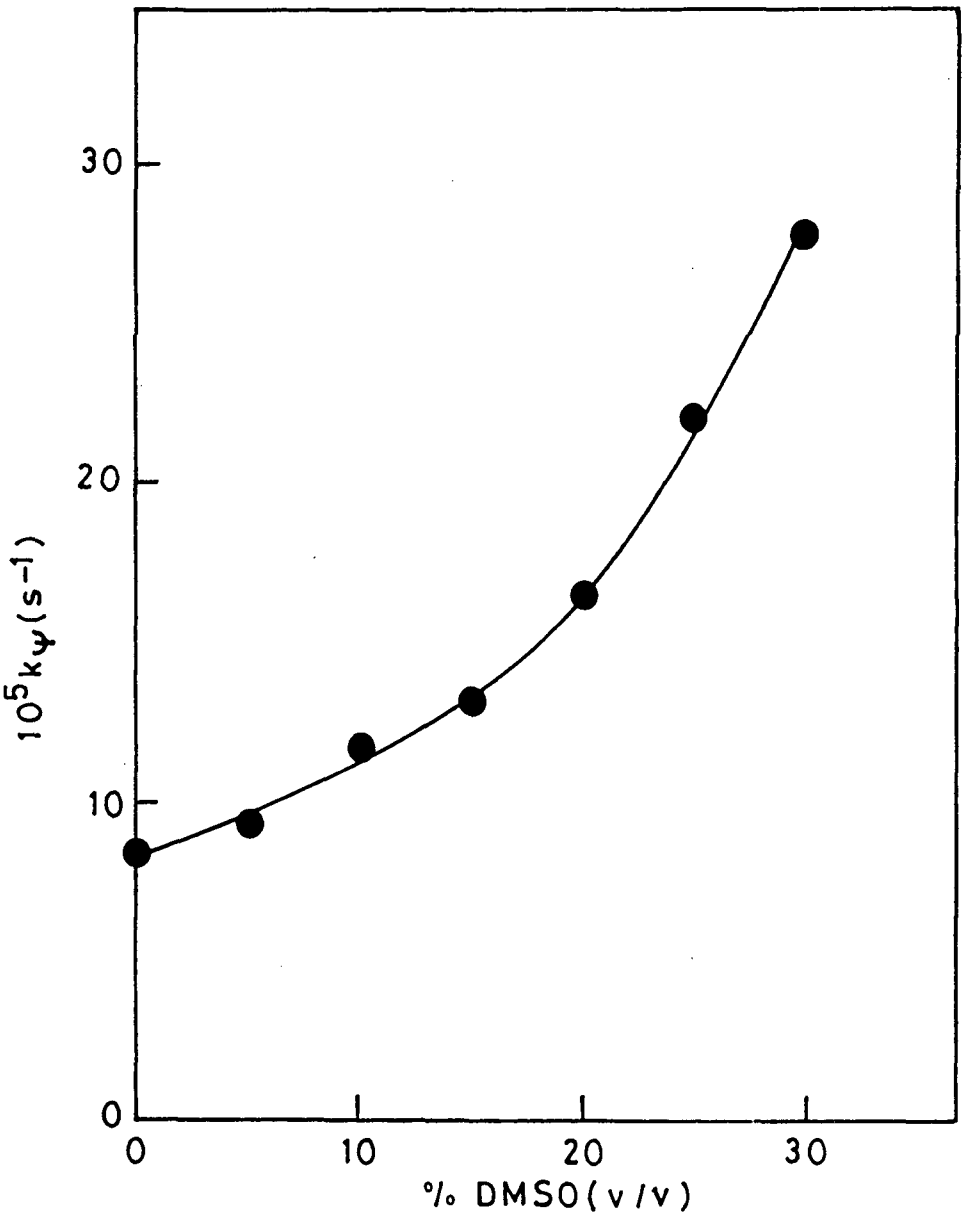


Fig. 3.27: Effect of DMSO on the reaction rate of glutamic acid with ninhydrin in presence of CTAB. *Reaction conditions* : $[\text{glutamic acid}]_T = 1.0 \times 10^{-4}$ mol dm^{-3}, $[\text{ninhydrin}]_T = 5.0 \times 10^{-3}$ mol dm^{-3}, $[\text{CTAB}]_T = 2.0 \times 10^{-2}$ mol dm^{-3}, pH = 5.0, temp. = 80 °C.

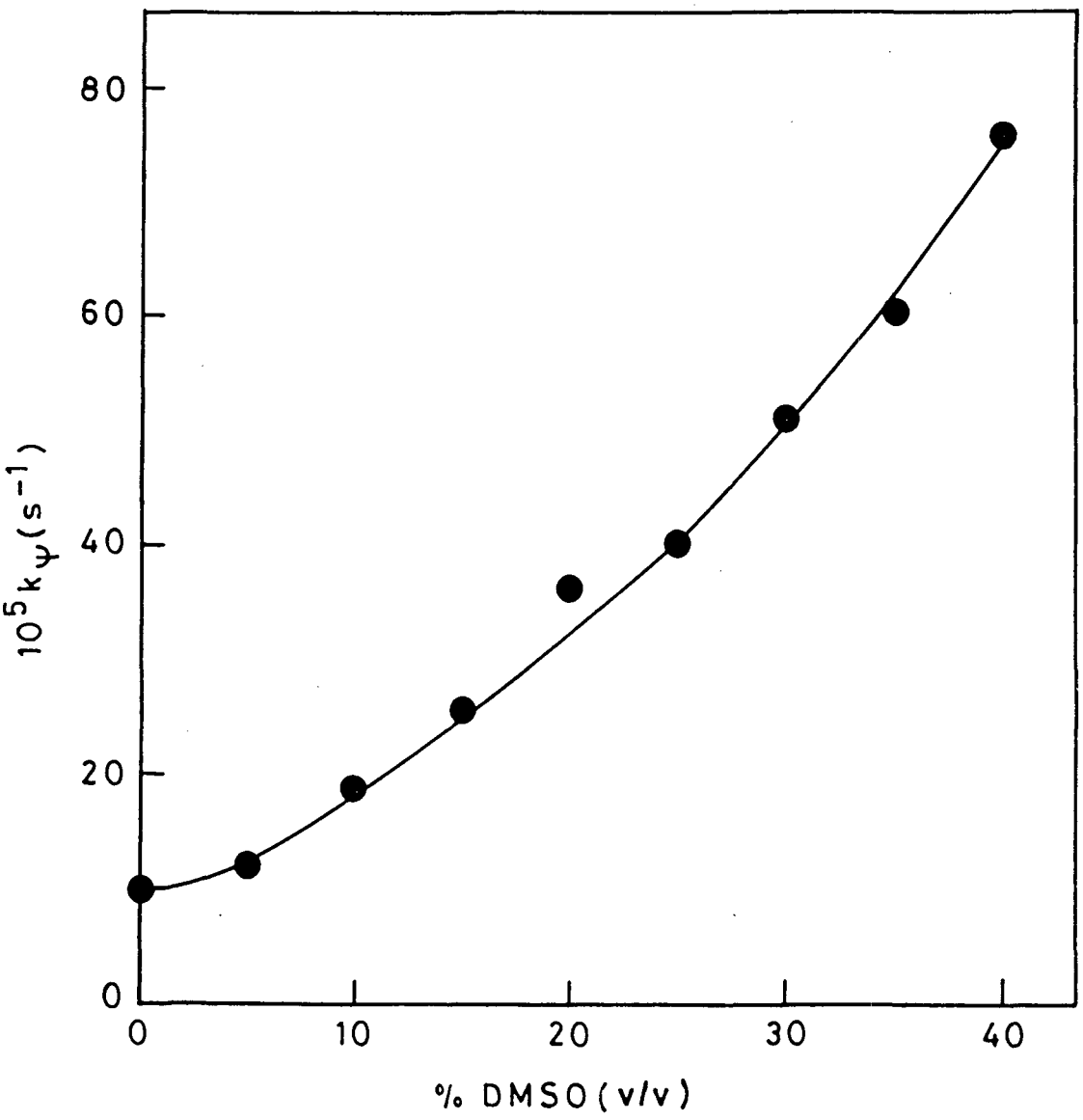


Fig. 3.28: Effect of DMSO on the reaction rate of arginine with ninhydrin in presence of CTAB. *Reaction conditions* : $[arginine]_T$ = 1.0 x 10^{-4} mol dm^{-3}, $[ninhydrin]_T$ = 5.0 x 10^{-3} mol dm^{-3}, $[CTAB]_T$ = 1.0 x 10^{-2} mol dm^{-3}, pH = 5.0, temp. = 80 °C.

TABLE 3.27

Effect of temperature on the *pseudo*-first-order rate constants (k_{obs} or k_{ψ}) in the absence and presence of CTAB for the reaction of alanine with ninhydrin.

Reaction conditions :

$[alanine]_T$ = 3.0×10^{-4} mol dm^{-3}

$[ninhydrin]_T$ = 5.0×10^{-3} mol dm^{-3}

pH = 5.0

Temperature (°C)	Aqueous		CTAB[a]	
	$10^5 k_{obs}$ (s^{-1})	$10^5 k_{cal}$[b] (s^{-1})	$10^5 k_{\psi}$ (s^{-1})	$10^5 k_{\psi cal}$[b] (s^{-1})
70	1.6	1.7	7.0	6.5
75	2.8	2.8	10.5	10.2
80	5.4	4.6	13.4	14.9
85	7.1	7.3	21.9	21.5
90	11.9	11.8	30.9	29.8
Parameters				
E_a (kJ mol^{-1})	103.9		77.1	
$\Delta H^{\#}$ (kJ mol^{-1})	101.0		74.2	
$-\Delta S^{\#}$ (J K^{-1} mol^{-1})	43.6		109.7	

[a][CTAB] = 1.0×10^{-2} mol dm^{-3}.

[b]calculated from Eq. (3.2).

TABLE 3.28

Effect of temperature on the *pseudo*-first-order rate constants (k_{obs}) for the reaction of methionine with ninhydrin.

Reaction conditions :

$[methionine]_T$ = 3.0×10^{-4} mol dm^{-3}

$[ninhydrin]_T$ = 5.0×10^{-3} mol dm^{-3}

pH = 5.0

Temperature (°C)	Aqueous	
	$10^5 k_{obs}$ (s^{-1})	$10^5 k_{cal}$[a] (s^{-1})
70	no reaction	
75	6.5	7.1
80	10.6	9.2
85	11.4	11.8
90	15.8	15.2
95	17.4	18.4
Parameters		
E_a (kJ mol^{-1})	52.6	
$\Delta H^{\#}$ (kJ mol^{-1})	49.6	
$-\Delta S^{\#}$ (J K^{-1} mol^{-1})	183.2	

[a]calculated from Eq. (3.2).

TABLE 3.29

Effect of temperature on the *pseudo*-first-order rate constants (k_{obs} or k_{ψ}) in the absence and presence of CTAB for the reaction of threonine with ninhydrin.

Reaction conditions :

$[threonine]_T$ = 2.0×10^{-4} mol dm^{-3}

$[ninhydrin]_T$ = 5.0×10^{-3} mol dm^{-3}

pH = 5.0

Temperature (°C)	Aqueous		CTAB[a]	
	$10^5 k_{obs}$ (s^{-1})	$10^5 k_{cal}$[b] (s^{-1})	$10^5 k_{\psi}$ (s^{-1})	$10^5 k_{\psi cal}$[b] (s^{-1})
70	3.6	3.9	9.5	9.8
75	5.6	5.5	13.1	12.7
80	7.8	7.8	17.4	16.3
85	10.5	11.0	19.9	21.1
90	15.1	15.5	27.5	27.1
Parameters				
E_a (kJ mol^{-1})	71.9		52.7	
$\Delta H^{\#}$ (kJ mol^{-1})	68.9		49.8	
$-\Delta S^{\#}$ (J K^{-1} mol^{-1})	130.0		178.0	

[a][CTAB] = 2.0×10^{-2} mol dm^{-3}.

[b]calculated from Eq. (3.2).

TABLE 3.30

Effect of temperature on the *pseudo*-first-order rate constants (k_{obs} or k_{ψ}) in the absence and presence of CTAB for the reaction of tyrosine with ninhydrin.

Reaction conditions :

$[tyrosine]_T$ = 1.0×10^{-4} mol dm^{-3}

$[ninhydrin]_T$ = 5.0×10^{-3} mol dm^{-3}

pH = 5.0

Temperature (°C)	Aqueous		CTAB[a]	
	$10^5 k_{obs}$ (s^{-1})	$10^5 k_{cal}$[b] (s^{-1})	$10^5 k_{\psi}$ (s^{-1})	$10^5 k_{\psi cal}$[b] (s^{-1})
70	6.2	6.2	8.7	9.4
75	7.8	8.3	11.6	11.9
80	11.5	11.1	14.3	15.0
85	15.6	14.9	18.5	18.9
90	18.7	20.0	22.3	23.8
Parameters				
E_a (kJ mol^{-1})	60.4		48.4	
$\Delta H^{\#}$ (kJ mol^{-1})	57.4		45.4	
$-\Delta S^{\#}$ (J K^{-1} mol^{-1})	159.5		191.1	

[a][CTAB] = 2.0×10^{-2} mol dm^{-3}.

[b]calculated from Eq. (3.2).

TABLE 3.31

Effect of temperature on the *pseudo*-first-order rate constants (k_{obs} or k_{ψ}) in the absence and presence of CTAB for the reaction of glutamic acid with ninhydrin.

Reaction conditions :

[glutamic acid]$_T$ = 1.0×10^{-4} mol dm^{-3}

[ninhydrin]$_T$ = 5.0×10^{-3} mol dm^{-3}

pH = 5.0

Temperature (°C)	Aqueous		CTAB[a]	
	$10^5 k_{obs}$ (s^{-1})	$10^5 k_{cal}$[b] (s^{-1})	$10^5 k_{\psi}$ (s^{-1})	$10^5 k_{\psi cal}$[b] (s^{-1})
70	no reaction	–	4.1	4.2
75	2.3	2.3	6.1	5.9
80	3.5	3.4	8.3	8.2
85	4.7	5.4	11.4	11.4
90	8.8	8.2	16.2	16.0
Parameters				
E_a (kJ mol^{-1})	89.1		69.5	
$\Delta H^{\#}$ (kJ mol^{-1})	86.2		66.5	
$-\Delta S^{\#}$ (J K^{-1} mol^{-1})	87.9		136.3	

[a][CTAB] = 2.0×10^{-2} mol dm^{-3}.

[b]calculated from Eq. (3.2).

TABLE 3.32

Effect of temperature on the *pseudo*-first-order rate constants (k_{obs} or k_{ψ}) in the absence and presence of CTAB for the reaction of arginine with ninhydrin.

Reaction conditions :

$[arginine]_T$ = 1.0×10^{-4} mol dm^{-3}

$[ninhydrin]_T$ = 5.0×10^{-3} mol dm^{-3}

pH = 5.0

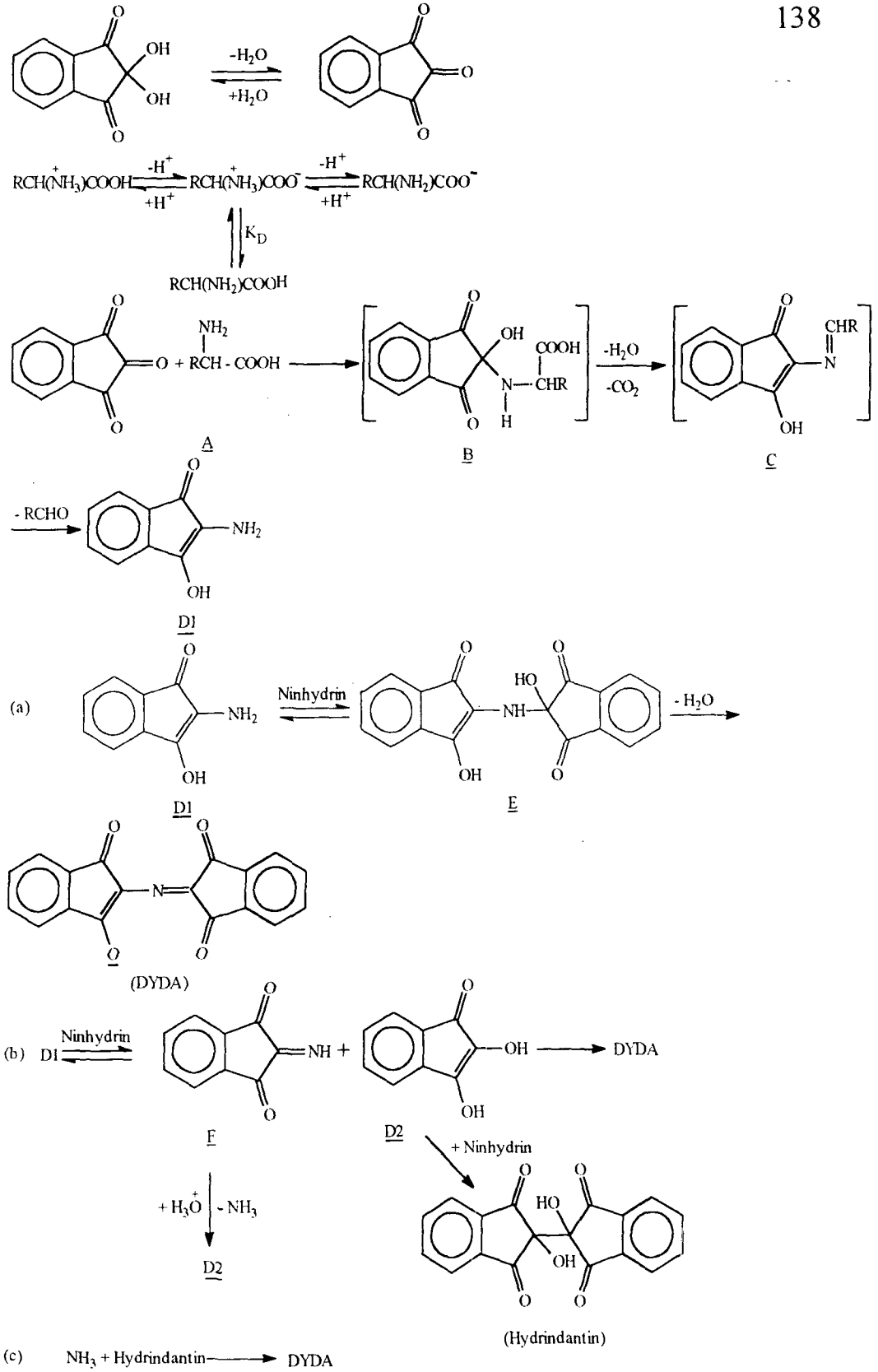
Temperature (°C)	Aqueous		CTAB[a]	
	$10^5 k_{obs}$ (s^{-1})	$10^5 k_{cal}$[b] (s^{-1})	$10^5 k_{\psi}$ (s^{-1})	$10^5 k_{\psi cal}$[b] (s^{-1})
70	2.0	2.0	6.0	6.0
75	3.2	3.0	8.7	8.0
80	4.3	4.3	10.0	10.7
85	5.6	6.3	13.5	14.2
90	10.0	9.2	19.9	18.9
Parameters				
E_a (kJ mol^{-1})	78.1		59.4	
$\Delta H^{\#}$ (kJ mol^{-1})	75.2		56.5	
$-\Delta S^{\#}$ (J K^{-1} mol^{-1})	117.3		162.6	

[a][CTAB] = 1.0×10^{-2} mol dm^{-3}.

[b]calculated from Eq. (3.2).

B. Discussion

Kinetics of the reaction between amino acids and ninhydrin in aqueous solution has been performed under the influence of various conditions such as temperature, pH, and reactant concentrations. It is well documented that all amino acids (except proline and hydroxyproline) on interaction with ninhydrin form a purple-coloured diketohydrindylidenediketohydrindamine (DYDA), popularly known as Ruhemann's purple; the summarized mechanism is provided in Scheme 3.1. Different amino acids react with different rates but all produce the same final product.[9,12-15,164,169] The condensation between the carbonyl group of ninhydrin and deprotonated amino group of amino acid takes place. The reaction starts through the attack of lone pair of electrons of amino nitrogen to the carbonyl group of ninhydrin to give a Schiff base (C, after decarboxylation) which is a characteristic of common addition-elimination reaction. This Schiff base is unstable and hydrolyses to give 2-amino-indanedione (D1) and an aldehyde. 2-Amino-indanedione, in turn, reacts with ninhydrin molecule to yield DYDA (route (a)). D1 gives ammonia (upon hydrolysis) and hydrindantin (route (b)). The yield of these products depends upon the reaction conditions (low pH and low temperature). At pH<5, the reaction proceeds chiefly by route (b), where ammonia is evolved quantitatively and no purple colour is produced. In solutions of pH>5, route (a) predominates and under these conditions, colour formation is the basis of the analytical method. Hydrindantin, if formed, reduces the yield of DYDA. The 2-imino-indanedione (F), upon hydrolysis gives ammonia, which may react with hydrindantin to give DYDA (route (c)). Therefore, the studies were performed such that reactions of the alternate routes (b) and (c) were


-H2O
+H2O
RCH(NH3)COOH
-H+
+H+
RCH(NH3)COO-
RCH(NH2)COO-
KD
RCH(NH2)COOH
NH2
RCH - COOH
COOH
CHR
N
H
-H2O
-CO2
A
B
C
- RCHO
NH2
OH
D1
(a)
Ninhydrin
HO
NH
E
(DYDA)
(b)
F
D2
+ Ninhydrin
+ H3O+
- NH3
DYDA
(Hydrindantin)
(c) NH3 + Hydrindantin ——→ DYDA

Scheme 3.1

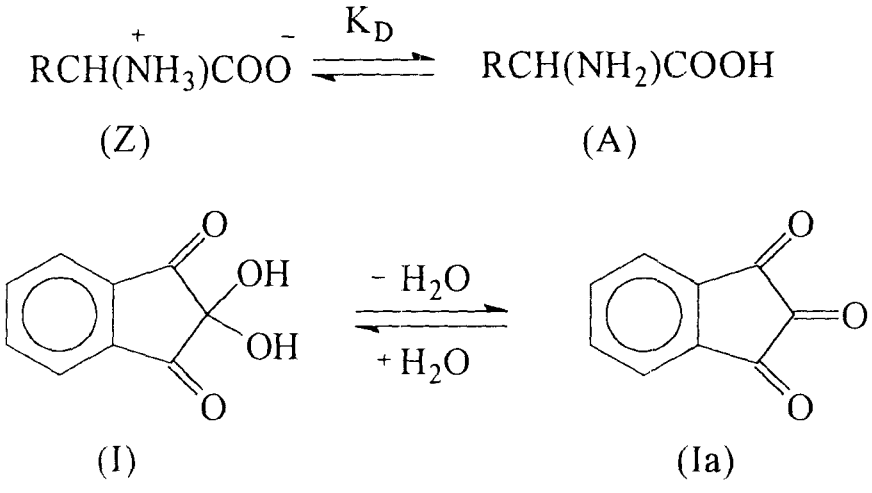
suppressed (almost completely) by carrying out experiments at elevated temperature (≥ 70 °C) and pH = 5.0 (formation of ammonia is negligible at pH ≥ 5).[12-14]

As regards the reaction in aqueous medium, the amount of reaction products (carbon dioxide, aldehyde, ammonia, hydrindantin and purple-coloured DYDA) have been found to depend upon the conditions of reaction medium, i.e., pH, temperature, [ninhydrin], etc.[6,12,13] It was also observed that the rate of evolution of CO_2 is fast in comparison to the rate of purple colour formation of DYDA. (At 80 °C, evolution of CO_2 was completed within 20 min while the intensity of purple colour was negligible within this time interval).[12,13] This confirmed that the reaction of ninhydrin with α-amino acid has two steps. The mechanism of first step is the formation of a Schiff base (C) which has a double-bonded nitrogen atom and the decarboxylation is considered to take place through unionized acid, possibly via formation of an unionized chelated ring structure. The decarboxylation step is not the rate-determining because, under the experimental conditions (pH 5.0), it is expected to be unimolecular and not subjected to steric hindrance. The hydrolysis of C to 2-amino-indanedione (D1) could not be rate controlling as well because rates should be governed by steric factors alone.[173] On the other hand, the reaction of D1 with ninhydrin also involves an addition-elimination type interaction leading to the formation of DYDA. The protonated amine group will decrease its nucleophilic character, while protonating the carbonyl group of ninhydrin will enhance the reaction. These two

conditions are affected oppositely by pH and, therefore, the maximum rate will be found at pH where not all of the $-NH_2$ group has been protonated and, at the same time, enough of the ninhydrin exists as its conjugate acid (keto form) to afford a reasonable reaction rate.

(a) ***Reaction of Ninhydrin with DL-Alanine, DL-Methionine, DL-Threonine and L-Tyrosine***

Under our experimental conditions, the zwitterionic form (Z) of amino acids (alanine, methionine, threonine and tyrosine) is the major existing species which is inactive towards the nucleophilic attack on the carbonyl carbon of ninhydrin[12,13,169] because the zwitterionic form has a positive charge on the nitrogen atom (the unshared electron pair of nitrogen is not available). Therefore, the neutral form of amino acids (A), which exists in equilibrium with the zwitterionic form[174], is the active species. On the other hand, ninhydrin also participates in the hydrated and dehydrated forms in the aqueous medium and the equilibrium states (Ia) and (I) are to be considered.



The aqueous medium results show that the rate constants are independent of the initial concentration of amino acids, indicating the order of the reaction in [amino acid] to be unity. The observed *pseudo*-first-order rate constants against [ninhydrin] plots show a non-linear behaviour, thus, indicating fractional order with respect to [ninhydrin].

On the basis of the observed rate law

$$r = k_{obs} [\text{amino acid}]_T \tag{3.3}$$

and the mechanism (Scheme 3.1), the rate Eq. (3.4) is derived :

$$k_{obs} = \frac{k\,K\,[nin]_T}{1 + K\,[nin]_T} \tag{3.4}$$

where $[nin]_T$ = total ninhydrin concentration. The data treatment was carried out by an alternative method using Eq. (3.5). The double-reciprocal plots resulted in straight lines, and thus confirmed the validity of the proposed mechanism.

$$\frac{1}{k_{obs}} = \frac{1}{k} + \frac{1}{k\,K\,[nin]_T} \tag{3.5}$$

The values of k and K were calculated from the intercepts (=1/k) and slopes (=1/kK) of the straight lines. The respective values of k and K are given in Table 3.33. The values of k and K were substituted in Eq. (3.5) to obtain the calculated values of rate constant (k_{cal}) (Tables 3.5, 3.8, 3.10, 3.12 and 3.14) in various kinetic runs. The close agreement between the observed and calculated values provides supporting evidence for the mechanism.

TABLE 3.33

Values of k and K obtained according to Eq. (3.5).

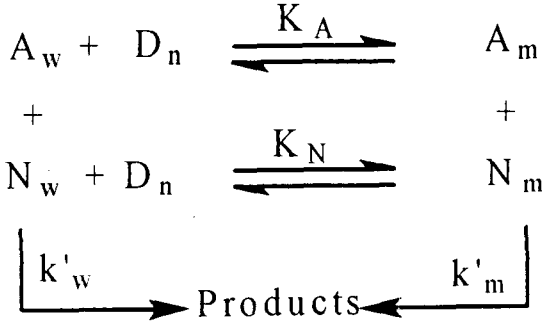
Amino acid	$10^4 k(s^{-1})$	$K(mol^{-1}\ dm^3)$
DL-Alanine	9.6	12.2
DL-Threonine	10.8	15.6
L-Tyrosine	4.8	59.8
L-Glutamic acid	5.5	13.8
L-Arginine	7.3	12.8

(b) ***Reaction of Ninhydrin with Glutamic Acid and Arginine***

It is well known that arginine is a basic amino acid whereas glutamic acid is an acidic amino acid. Therefore, at pH 5.0 the predominent species of the former will be positively charged $H_3\overset{+}{N}-\underset{\underset{NH}{\|}}{C}-NHCH_2CH_2CH_2CH(NH_2)COOH$ while that of the latter will be negatively charged ($^-OOC\text{-}CH_2CH_2CH(NH_2)COOH$). Irrespective of the charge, it is the unprotonated α-NH_2 which should be available for the condensation. Hence in these cases also the mechanism of reaction would remain the same.[14]

Now we take into account the kinetic data obtained in micellar systems. Figs. 3.13-3.17 show that the observed *pseudo*-first-order rate constants increase upon increasing the [CTAB] at low surfactant concentrations that reach a maximum value. The profile shape is perfectly general being a common characteristic of reactions catalyzed by micelles.[148,149,175]

The reaction rate in the presence of CTAB micelles may be discussed in terms of pseudophase model of the micelles developed by Menger and Portnoy[148] and modified by Bunton[149,159] and Vera and Rodenas.[176] Under the experimental conditions of this study the reaction scheme in the presence of CTAB micelles may be given as



Scheme 3.2

A is the amino acid whose binding constant to the micelle, K_A, is written in terms of micellized surfactant :

$$K_A = \frac{[A_m]}{[A_w]\,[D_n]} \tag{3.6}$$

The *pseudo*-first-order rate constants, k'_w and k'_m, in aqueous and micellar *pseudo*-phases are given by:

$$k'_w = k_w\,[N_w] \tag{3.7}$$

$$k'_m = k_m\,[N_m]/[D_n] = k_m\,m_N^S \tag{3.8}$$

where k_w and k_m are the second-order rate constants and m_N^S is the mole ratio of ninhydrin bound to the micellar head group. From Scheme 3.2 rate equation (3.9) is derived:

$$k_\psi = \frac{k_w\,[N]_T + (K_A k_m - k_w)\,m_N^S\,[D_n]}{1 + K_A\,[D_n]} \tag{3.9}$$

Values of mol ratio m_N^S (= $[N_m]/[D_n]$) were estimated by considering the equilibrium

$$N_w + D_n \underset{}{\overset{K_N}{\rightleftharpoons}} N_m$$

$$K_N = \frac{[N_m]}{[N_w]\,([D_n] - [N_m])} \tag{3.10}$$

and the mass balance equation (3.11) for the ninhydrin, i.e.,

$$[N]_T = [N_w] + [N_m] \quad (3.11)$$

Upon solving Eqs. (3.10) and (3.11), a quadratic equation (3.12) results, which was solved for $[N_m]$ with the help of a computer program with K_N as an adjustable parameter. m_N^S was then calculated with the help of Eq. (3.8).

$$K_N [N_m]^2 - (1+K_N [D_n] + K_N [N]_T) [N_m] + K_N [D_n] [N]_T = 0 \quad (3.12)$$

In order to determine k_m and K_A kinetically we need the cmc under kinetic conditions which were determined conductimetrically (see Experimental). For a given value of cmc, the k_m and K_A were calculated from Eq. (3.9) using a non-linear least squares technique. Such calculations were carried out at different presumed values of K_N. The best fit values are recorded in Table 3.34. The fitting of the calculated data (K_A, k_m and K_N) to Eq. (3.9) is evident from the calculated values of rate constants, $k_{\psi cal}$, shown in Tables 3.16 - 3.20.

In order to confirm the Scheme 3.1 mechanism, effect of variables on the rate constants were seen in presence of constant [CTAB]. It was found that the reaction follows the same first- and fractional-order kinetics with respect to [amino acid] and [ninhydrin]. Thus, we can conclude that the reaction mechanism remains the same in presence of CTAB micelles as that in the aqueous medium with all possible

TABLE 3.34

Values of rate parameters (k_m, k_2^m, k_w, and k_w/k_2^m) and binding constants (K_A, K_N) for the reaction of ninhydrin and amino acids in micellar media.

Reaction conditions :

$[ninhydrin]_T$ = 5.0×10^{-3} mol dm^{-3}

pH = 5.0

Temperature = 80 °C

Parameters and constants	Amino acids				
	Ala[a]	Thr[b]	Tyr[c]	Glu[c]	Arg[c]
$10^3 k_m$ (s^{-1})	2.8	5.6	5.0	1.95	2.5
$10^4 k_2^m$ ($mol^{-1}dm^3s^{-1}$)[d]	3.9	7.8	7.0	2.73	3.5
$10^3 k_w$ ($mol^{-1}dm^3s^{-1}$)	10.8	15.6	23.0	7.0	8.6
k_w/k_2^m	27.6	20.1	32.9	25.6	24.6
K_A ($mol^{-1}dm^3$)	108	82	58	64	78
K_N ($mol^{-1}dm^3$)	69	60	60	72	53

[a][Alanine] = 3.0×10^{-4} mol dm^{-3}.

[b][Threonine] = 2.0×10^{-4} mol dm^{-3}.

[c][Amino acid] = 1.0×10^{-4} mol dm^{-3}.

[d]Second-order rate constants (k_2^m) are based on Eq. (3.13).

intermediary situations. In the micellar medium the reaction of both $(A)_w$ and $(A)_m$ with $(N)_w$ and $(N)_m$ takes place. The enhancement of rate in presence of cationic micelles could then be attributed to the stabilization of intermediate C (i.e., the Schiff base) on the positively charged micellar surface, thereby increasing the concentration of the intermediate in the Stern layer. The presence of π-electrons in ninhydrin[164] increases the possibility of its partitioning between water and positively charged micelles.[177] Therefore, both the reactants get effectively incorporated/associated into the aqueous surface of the micelles (i.e., the Stern layer – considered to be the usual sight of ionic micelle-mediated organic reactions). Thus, the overall increase of reaction rate is due to concentrating both the reactants in the micellar reaction zone.

The second-order rate constant k_m is expressed in reciprocal seconds (Table 3.34). It cannot be compared directly with the rate constant in water (k_w, mol^{-1} dm^3 s^{-1}). In order to convert k_m to conventional units, Bunton[159] used the volume element for reaction within the micellar *pseudo*-phase. The Stern layer volume of one mole of CTAB is about 0.14 dm^3, the conventional second-order rate constant (k_2^m, mol^{-1} dm^3 s^{-1}) for reaction in the Stern layer is then given as in Eq. (3.13).

$$k_2^m = 0.14\ k_m \qquad (3.13)$$

Generally, $k_w > k_2^m$ for many bimolecular reactions in aqueous and micellar *pseudo*-phases.[150,178] However, there are many examples in which k_2^m are similar to magnitude with k_w.[159] It has been assumed that overall

rate enhancements of bimolecular reactions are due to the association/ incorporation of both the reactants in the small volume of the micellar *pseudo*-phase. Micellar surfaces are water rich but are less polar than water and do not provide a uniform reaction medium because micelle is a porous cluster with rough surface and deep water filled cavities.[179] The different values of k_w/k_2^m for different amino acids may simply reflect the above facts.

Effect of Temperature

The effect of temperature on the CTAB-catalyzed reactions of amino acids with ninhydrin in presence of constant CTAB was used to evaluate activation parameters. The results are recorded in Tables 3.27, 3.29-3.32. Comparing the values with those obtained in aqueous medium (Tables 3.27, 3.29-3.32), we find that the presence of cationic micelles catalyze the reaction of amino acids with ninhydrin and lower the $\Delta H^{\#}$ with more negative $\Delta S^{\#}$. This lowering occurs not only through the adsorption of both the reactants on the micellar surface but also through stabilization of the transition state.[6] A meaningful mechanistic explanation of the apparent values of $\Delta H^{\#}$ and $\Delta S^{\#}$ is not possible because the k_ψ does not represent a single elementary kinetic step; it is a complex function of true rate, binding and ionization constants.

The fitting of the observed k_ψ-values at different temperatures to Eyring equation for the micellar media indicates that the sensitivity of micelle structure to temperature is kinetically unimportant.

Effect of Solvents

Addition of small amounts of water-soluble organic solvents markedly increase the rate as well as intensity of the colour (Tables 3.21-3.26, Figs. 3.18-3.23). This unique behaviour of organic solvents towards the ninhydrin-amino acid reaction may be explained by the fact that as the solvent volume increases, the volume of water decreases in a given set of experiments, resulting in a decrease of the rate of hydrolysis (cf. Scheme 3.1). Thus, with the increasing organic solvent content, the side reaction is progressively blocked. Secondly, Ruhemann's purple is highly soluble in organic solvents[9,12,13,170,180], thereby imparting increased intensity. A combined presence of DMSO and surfactant shows a synergistic effect (Figs. 3.24-3.28), which could be due to blockage of side reaction and preconcentration of reactants in a small volume of the micellar surface region.

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